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NUCLEAR PHENOMENA OF SEXUAL REPRODUCTION IN ALGÆ[1]

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CYTOLOGICAL investigations among the algæ upon the processes of gametogenesis and the developments that follow upon the fusion of gametes have not been numerous and are all of comparatively recent date. The difficulties of obtaining and handling material in critical stages require great patience, but the importance of the problems has attracted attention to a field of enquiry in which considerable progress has already been made and which offers some of the most attractive problems for cytological investigation. There has been a very marked advance in our understanding of the Rhodophyceæ and Phæophyceæ, but the Chlorophyceæ have so far received little attention.

The advance has been chiefly in our interpretation of certain life histories in which complex developments follow upon the phase of sexual reproduction. The publication in 1894 of Strasburger's paper on "The Periodic Reduction of the Number of Chromosomes in the Life History of Living Organisms"[2] marked the beginning of that closer analysis of the life histories of the higher plants which presents the strongest support for the

[1] A paper read by invitation before the Botanical Society of America, Boston, December 30, 1909.

[2] *Ann. of Bot.*, Vol. VIII, p. 281, 1894.

theory of antithetic alternation of generations. The higher plants were naturally the first which were subjected to critical cytological investigation and the structure and behavior of the nuclei through various stages of ontogeny have been studied in an increasing number of forms representative of the most important groups. Later came the pioneer work of this character upon the thallophytes which has given such rich returns in our appreciation of the significance of the critical stages in the complex life histories of certain types of the Ascomycetes, Uredineæ, Phæophyceæ and Rhodophyceæ.

It is the writer's part in this symposium to outline the advances that have been made in cytological research on the sexual reproduction of the algæ, and such enquiry, for a number of reasons, necessarily leads at once to the consideration of life histories with reference to cytological detail. From studies upon the bryophytes, pteridophytes and spermatophytes we have learned that in these groups every sexual nuclear fusion marks the beginning of a distinct phase in the life history, the sporophyte, the nuclei of which carry the double, or diploid, number of chromosomes characteristic of the species. Furthermore, we know that the sporophytic phase comes to an end with the numerical reduction of the chromosomes to the original, or haploid, number peculiar to the gametophyte. This significant period of chromosome reduction is associated in the bryophytes and pteridophytes with the formation of spores, in the spermatophytes with the formation of pollen grains and embryo sacs. Every sexual fusion, then, carries with it a prospective period of chromosome reduction.

Since such is the history for the higher plants we have every reason to expect that each sexual nuclear fusion among the thallophytes with the resulting doubling of the chromatin content in the zygote would be followed sooner or later by reduction phenomena. It becomes a matter of importance to determine when the latter event occurs, since between the time when the gametes fuse and that of

chromosome reduction there is always the possibility of an intercalated sporophytic phase in the life history, the development of which will involve the mitoses of nuclei that carry twice the number of chromosomes characteristic of the sexual plant. From this point of view let us then consider in the order of the Chlorophyceæ, Phæophyceæ and Rhodophyceæ some of the recent cytological research which has made much clearer the significance of the life histories of certain important types.

There are in the life histories of a number of the green algæ certain outward signs that would lead one to suspect that the period of chromosome reduction in these forms takes place just previous to or during the germination of the zygospores or oospores. Thus the formation of several zoospores within the zygote of *Ulothrix* and *Hydrodictyon,* and of four zoospores in the oospore of *Œdogonium* at once excites suspicion that the first mitosis in these sexually formed cells is a reduction division. Still more striking is the phenomena described in the zygospores of the Conjugales where the fusion nucleus by two mitoses gives rise to four daughter nuclei, three of which in *Spirogyra* and two of which in the desmids *Closterium* and *Cosmarium* break down. Such behavior, involving a sacrifice of protoplasmic structure and energy, is not easily understood except that it be related to important events of ontogeny and phylogeny. Any one familiar with the cytology of spore formation in the higher plants or with oogenesis in animals would at once suspect that the zygospore of the Conjugales was the seat of reduction phenomena.

That this is really true has been shown by recent work of Karsten[3] who has followed the chromosomes of *Spirogyra jugalis* through the two mitoses within the zygospore. Karsten in agreement with Berghs reached the conclusion that the chromosomes in the resting nucleus

[3] Karsten, G., 1908, "Die Entwicklung der Zygoten von *Spirogyra jugalis* Ktzg.," *Flora,* Vol. XCIX, p. 1, 1908.

of *Spirogyra* lie in the substance of the large nucleole.[4] He was able by the proper extraction of stain to differentiate fourteen bodies in the gamete nucleus which corresponded in number to the fourteen chromosomes that may be readily counted for this species in later mitoses. The union of the gamete nuclei in the zygospore involves the fusion of the two nucleoles to form a dense homogeneous structure in preparation for a characteristic association of the two sets of chromosomes, one derived from each parent.

The approach of the first mitosis is indicated by changes within the fusion nucleole where a granular structure begins to appear, accompanied by irregularities in its form. As these changes proceed an assemblage of deeply staining bodies becomes evident which in later stages are readily identified as chromosomes. There are twenty-eight of these grouped in fourteen pairs, an arrangement which is characteristic of a phase of chromosome reduction that follows synapsis and which signifies that the chromosomes are to be distributed in two sets of equal numbers by the following mitosis. This distribution occurs in *Spirogyra* and the second mitosis in the zygospore is concerned with the reduced, or haploid, number of chromosomes. Thus the double, or diploid, number is present only in the fusion nucleus; the numerical reduction takes place with the first mitosis. There are no nuclear divisions in which the diploid number of chromosomes is passed on, and assuming that the sporophyte generation is characterized by nuclear divisions with the diploid number of chromosomes, there is no sporophytic phase in this life history.

So close is the parallelism between the events of reduction in *Spirogyra* and those of higher plants that Karsten even reports evidence for the premature division of the chromosomes during the first mitosis in preparation for

[4] B. F. Lutman has, however, recently reported (*Science*, Vol. XXXI, p. 633, 1910) that the chromosomes of *Closterium* are formed from a spirem which has its origin in the fine reticulum around the nucleole.

the second division, a phenomenon very characteristic of the reduction divisions of higher plants. The degeneration of three of the four nuclei which result from the two mitoses within the zygospore of *Spirogyra* is of course an illustration of that conservation of material for a single reproductive cell which finds its analogy in the history of development of certain megaspores at the expense of their neighbors and in the maturation of the animal egg.

It seems probable that studies upon the germinating zygospores and oospores of other green algæ will establish them to be the seat of reduction phenomena similar to that described above, although such investigations are beset with many technical difficulties. It is to be hoped that we may soon have information on these conditions in such types as *Œdogonium, Sphæroplea, Chara* and *Vaucheria.* The complexities reported in the formation of auxospores among the diatoms take on new interest in relation to the conditions in *Spirogyra.* In none of these forms have we at present any reason to expect the presence of a sporophytic generation.

Coleochæte has long been regarded as a type of peculiar interest chiefly perhaps because its oospore on germination gives rise to a small group of cells, each of which develops a zoospore. It is not strange that this cellular structure, a phase intercalated between two sexual plants, should have been compared with the simplest types of sporophytes in the liverworts, and that in attempts to bridge the gap between the thallophytes and bryophytes *Coleochæte* should have been brought forward as illustrating the primitive beginnings of a sporophyte generation.

A cytological study by Allen[5] on the germination of the oospore of *Coleochæte,* however, presents evidence that the first mitosis in the oospore is a reduction division and that consequently the later mitoses can not be sporo-

[5] Allen, C. E., 1905, "Die Keimung der Zygote bei *Coleochæte,*" *Ber. deut. bot. Gesell.,* Vol. XXIII, p. 286, 1905.

phytic in character. Allen found that when the fusion nucleus made preparations for the first mitosis the chromatic material previously in the form of an irregular network gathered at one side of the nucleus in the manner characteristic of synapsis. There was evidence of a pairing of the chromatic threads during the process of synapsis which is interpreted by this author as indicating a pairing of the chromosomes from the two gamete nuclei. Following synapsis the chromatic material becomes distributed as a coiled thread from which the chromosomes are differentiated, that in a later stage are found scattered through the nuclear cavity. A count of the chromosomes at this stage indicated that the number thirty-two is the double or diploid number for the species studied. Some of the chromosomes were clearly associated in pairs, a condition that becomes more evident in later stages when at metaphase of the first mitosis the now much condensed chromosomes are assembled at the equatorial plate. In this mitosis the chromosomes are short and thick, in sharp contrast to the long narrow bent rods which are characteristic of the second mitosis. Allen then bases his conclusion that the first mitosis in the oospore is a reduction division chiefly on the presence of a stage similar to synapsis preceded by a long resting period, and on peculiarities in the form and grouping of the chromosomes, which peculiarities are similar to those of the heterotypic division in higher plants.

It would seem then that *Coleochæte* can no longer be considered as a type very helpful in considerations of the origin of the sporophyte or suggestive of affinities with the archegoniates. With respect to the latter point the simple unicellular character of the sexual organs offers further difficulties to a relationship to the archegoniates, since the multicellular archegonia and antheridia of this last group apparently require an origin from a multicellular type of sexual organ such as is best illustrated among living forms by the plurilocular sporangia and

gametangia of the Phaeophyceae. The writer has discussed this problem in a paper on "The Origin of the Archegonium,"[6] and advanced the view that the archegonium and antheridium arose from some type of plurilocular sporangium or gametangium through the differentiation, in response to terrestrial life habits, of a sterile protective envelope around the gametes. Schenck in a contribution "Ueber die Phylogenie der Archegoniaten und der Characeen"[7] accepts the above view and even argues for a direct descent of the archegoniates from the Phæophyceæ, carrying the speculation far beyond the point with which the writer is in agreement. A discussion of the difficulties which face Schenck's hypothesis would, however, lie outside of the province of this paper.[8]

Assuming that the sporophyte is normally characterized by mitoses concerned with the diploid number of chromosomes, the results of the recent research on the Chlorophyceæ make it appear probable that none of the known living types present an alternation of generations even in its simplest form. The striking differences between the Chlorophyceæ and the simplest archegoniates have become more strongly emphasized by the latest research.

Passing to the Phæophyceæ we shall have to consider cytological conditions and life histories very much more complicated than any that are known for the Chlorophyceæ. Only the specialist among the algæ can fully appreciate the diversity of the types and lines of development in the large assemblage of forms termed the brown algæ. The chief groups exhibit greater differences among themselves than do the divergent lines among the green algæ. Unfortunately our information on the cytology of reproduction in the Phæophyceæ is limited to three groups, the Dictyotales, Fucales and Cutleriaceæ, groups which occupy isolated positions of problematical relationship to the lower forms of the Phæophyceæ.

[6] *Ann. of Bot.*, Vol. LXVII, p. 477, 1903.
[7] *Engler's Botan. Jahrbücher*, Vol. XLII, 1908.
[8] See review by Davis, AMER. NAT., Vol. XLIII, p. 107, 1909.

We have from the investigations of Williams[9] an account of the cytology of *Dictyota* throughout the critical phases of its life history. There are three forms of this alga, the male and female sexual plants and an asexual plant which develops spores in groups of four (tetrads) within a tetrasporangium. Earlier research of Mottier indicated clearly that the tetraspore mother-cell is the seat of chromosome reduction, and Williams by studies of the sexual plants as well as the asexual was able to present convincing cytological evidence of an alternation of generations.

Williams found that the sexual plants were characterized by nuclei with sixteen chromosomes and that this number was passed on to the eggs and sperms respectively. Fertilization takes place following the discharge of the gametes, which have the peculiarity of being developed in fortnightly crops, "each crop being initiated a little before the lowest neap tide, and arriving at maturity about the period of the highest succeeding spring tide". The motile sperms gather about the eggs which for a short time exert a strong chemotactic influence. Following the union of the gamete nuclei, a second nucleole appears in the fusion nucleus which Williams believes to be the chromatin brought by the sperm. The first mitosis in the egg presents thirty-two chromosomes at the equatorial plate, and this is believed to be the first mitosis of a sporophyte generation represented by the asexual plant and terminating with the development of the tetraspores.

The spindle poles of the first mitosis in the eggs appear to arise by the division of an aster the two poles of which separate until they come to lie on opposite sides of the nucleus. It is an interesting fact that unfertilized eggs begin a parthenogenetic development, but the spin-

[9] Williams, J. Lloyd, 1904, "Studies on the *Dictyotaceæ*." I., "The Cytology of the Tetrasporangium and the Germinating Tetraspore," *Ann. of Bot.*, Vol. XVIII, p. 141, 1904. II., "The Cytology of the Gametophyte Generation," *Ann. of Bot.*, Vol. XVIII, p. 183, 1904.

dles of the first mitosis are multipolar and the chromosomes are distributed irregularly to a cluster of small nuclei. It would seem then either that the sperm brings to the egg a directive element in the form of an aster, which gives polarity to the fusion nucleus, or that in the absence of fertilization the egg is unable to develop the mechanism necessary for a normal mitosis.

It is the purpose of this paper to consider the nuclear phenomena of sexual reproduction among the algæ in the broadest sense, and this must involve the consideration of any sporophytic generation when present. It has become clearly established by the cytological research of recent years that the sporophytic phase in a life history is a period during which one of the final ends of a sexual nuclear fusion, the intimate association of the sets of parental chromosomes, is delayed and in consequence the mitoses of this period deal with double the number of chromosomes present in the sexual plants. Williams found in the case of *Dictyota* that the mitosis which cuts off the tetraspore mother-cell presented the same large number of chromosomes as the first division of the egg, *i. e.*, twice the number characteristic of the sexual plants. The inference is clear that the vegetative mitoses throughout the development of the asexual plant must be sporophytic in character, and that this generation must have developed from the fertilized egg.

With respect to the two mitoses within the tetrasporangium, the main events are evidently those characteristic of a numerical reduction of the chromosomes. There is a stage of synapsis in which a long thin spirem is found closely coiled in knots against the nuclear membrane. Following this comes a loosening up of the spirem and the differentiation of sixteen chromosomes, the reduced number. The form of the chromosomes, which are loops and closed rings, indicates at once to one familiar with the peculiarities of the heterotypic mitosis that these structures are really pairs of sporophytic chromosomes (bivalent chromosomes) in close association.

The members of each pair are separated by the first mitosis which thus distributes the thirty-two chromosomes in two sets. The second mitosis (homotypic) presents sixteen simple chromosomes in the form of bent rods, similar to those found in the gametophytes. The cytological evidence justifies the conclusion that the tetraspores give rise to the sexual plants and that the fertilized egg must develop the asexual plant, and thus is established an alternation of generations.[10]

The peculiarities of the life history of *Fucus* in relation to the life histories of plants in general are most striking. It became evident from the studies of Strasburger in 1897 and of Farmer and Williams in 1896–98 that the mitoses within the oogonium presented only half the number of chromosomes which were present in the vegetative cells of the Fucus plant. This placed the period of chromosome reduction just previous to the differentiation of the gametes, a condition that is not known in any other group of the thallophytes. To bring such a life history into relation with the prevailing conditions in the lower plants becomes a most interesting problem of plant morphology and phylogeny.

We have recently had from Yamanouchi[11] a much more detailed account of the reduction processes in *Fucus* than the descriptions of the earlier writers. The nucleus within the young oogonium passes through a remarkably clearly defined stage of synapsis during which chromatic threads, derived from a reticulum, become arranged in loops that are gathered together closely attached at one

[10] Since the above was written W. D. Hoyt (*Bot. Gaz.*, Vol. XLIX, p. 55, 1910) has announced his success in raising from the fertilized eggs of *Dictyota dichotoma* fruiting plants which proved to be asexual (tetrasporic) and from the tetraspores sexual plants both male and female. Thus the conclusions based upon cytological evidence have been sustained by cultural studies. Such investigations are especially difficult to conduct in the case of marine algæ, but it is to be hoped that Hoyt's success with *Dictyota* will lead to further studies of this character, especially upon forms of the Rhodophyceæ.

[11] Yamanouchi, S., 1909, "Mitosis in *Fucus*," *Bot. Gaz.*, Vol. XLVII. p. 173, 1909.

side of nuclear membrane. Close to this point but outside of the nuclear membrane, there is developed an accumulation of kinoplasm from which is differentiated an aster with a chromosome, so that the nucleus during synapsis exhibits strongly marked polarity.

The chromatic loops extend into the nuclear cavity and sections show them to be thirty-two in number. After a period of condensation each loop is transformed into a pair of chromosomes by the separation of the arms at the bend. The pairs of chromosomes then become scattered through the nuclear cavity (diakinesis). The interpretation of this history is that the sixty-four somatic chromosomes of the *Fucus* plant are arranged end to end on the spirem and become associated in thirty-two pairs through the formation of the loops. Meanwhile a second aster appears, apparently arising *de novo,* generally at some distance from the first, and the two asters establish the poles of the first spindle. The thirty-two pairs of chromosomes are gathered at the equatorial plate and the members of the pairs distributed in two sets, thus affecting a numerical reduction by one half of the sixty-four somatic chromosomes.

There is apparently no premature division of the chromosomes in preparation for the succeeding division, as is generally the case in the heterotypic mitosis. The second and third divisions in the oogonium are similar in all essentials to typical mitoses. They deal, of course, with thirty-two chromosomes, the haploid number, which divide lengthwise during the metaphase of each mitosis. There is a long period of rest following the second mitosis. The first mitosis in the antheridium is likewise a reduction division similar to that in the oogonium and need not be described in this connection.

The nucleus of the unfertilized egg exhibits no evidence of polarity, but following the entrance of the sperm, according to Yamanouchi, an aster with a centrosome becomes at once evident. A second centrosome with radiations appears later at the point where the sperm

fuses with the egg nucleus. These two asters establish the spindle poles of the first mitosis, which presents sixty-four chromosomes. The chromatin of the two gametes is indistinguishable in the fusion nucleus.

The interpretation of the life history of *Fucus* with its phase of chromosome reduction so close to the differentiation of the gametes involves great difficulties. Strasburger has long held the thallus of this plant to be a sporophyte generation, assuming that the gametophyte phase is represented by the third mitosis in the oogonium and the few antheridial mitoses which follow the reduction division. He would then regard the antheridia and oogonia to be derived not from primitive sexual organs, but from sporangia corresponding to the tetrasporangia of *Dictyota*. This view involves a reversal of the relations between the gametophyte and sporophyte generations usual among the algæ, since it supposes the almost complete suppression of the gametophyte. However interesting and suggestive is this interpretation, it can hardly be considered other than a speculation until we know more of the probable phylogeny of the *Fucales*.

It has been evident for a number of years that the life history of the Cutleriaceæ probably involved two phases represented by sexual and asexual plants, respectively, and there has accumulated much evidence from the studies of Reinke, Falkenberg, Sauvageau, Church and others indicating that these phases present a true alternation of generations. The forms most studied have been *Cutleria multifida* with a large much branched thallus which develops gametes, and *Aglaozonia reptans,* a small crustaceous alga which reproduces by zoospores. On the germination of the zygotes and zoospores of these types forms are produced which very shortly take on vegetative characters not of the parent plants, but from the zygotes arise *Aglaozonia*-like sporelings and from the zoospores sporelings totally unlike *Aglaozonia,* but with characteristics of *Cutleria*. Upon this behavior chiefly have been

based the suggestions of an alternation of generations in the Cutleriaceæ.

A preliminary paper by Yamanouchi[12] on the above named species presents cytological evidence in support of the theory that they are sexual and asexual phases, respectively, of the same life history, with the relation one to the other of gametophyte and sporophyte. The vegetative mitoses of *Cutleria multifida* and those leading to the formation of the male and female gametes show uniformly twenty-four chromosomes. The zygote develops at once into a sporeling, the nuclei of which have forty-eight chromosomes. There is thus no reduction of the chromosomes at the time of the germination of the zygote, and the sporeling which results must be considered as sporophytic in character.

The vegetative mitoses of *Aglaozonia reptans* exhibits forty-eight chromosomes, but the nuclear divisions within the sporangia are quite different. Following the differentiation of a sporangium, the nucleus passes through a stage of synapsis in which the chromatic spirem is arranged in a series of loops from which are developed twenty-four bivalent chromosomes. The first division in the sporangium is therefore a heterotypic mitosis. Later mitoses in this cell exhibit the reduced number of chromosomes. Since sporogenesis in *Aglaozonia* is accompanied by reduction phenomena, there must be in its life history a sexual phase of which it is the sporophyte generation, and the number of chromosomes as well as other details of cell structure present strong cytological evidence that the gametophyte is *Cutleria*. Such evidence supporting the conclusions of the earlier writers based on the seasonal habits of *Cutleria multifida* and *Aglaozonia reptans* and on the structure of the respective sporelings, makes as strong a case for an alternation of generations in the Cutleriaceæ as is possible short of the actual cultivation of these algæ from zygotes and zoospores to fruiting maturity.

[12] Yamanouchi, S., 1909, "Cytology of *Cutleria* and *Aglaozonia*," *Bot. Gaz.*, Vol. XLVIII, p. 380, 1909.

These results from studies on the three most highly differentiated groups of the Phæophyceæ (Dictyotales, Fucales and Cutleriaceæ) indicate how complex has been the evolutionary history of the divergent phyla assembled under this name, and how difficult are likely to be the problems of tracing their relationship to lower groups of the brown algæ.

Perhaps in no assemblage of the algæ have the cytological studies of recent date given such striking results as in the Rhodophyceæ. Our understanding of the life history of these forms has been quite revolutionized by the research of three investigators, Wolfe, Yamanouchi and Lewis.

Wolfe[13] from studies on *Nemalion* determined for this type that the sexual organs are not simple uninucleate cells, since the trichogyne possesses a nucleus in addition to that in the carpogonium and the sperm is binucleate and the homologue of the antheridium. The cells of the cystocarp following the fertilization of the carpogonium have nuclei with approximately sixteen chromosomes which is double the number present in the vegetative cells of the parent plant. The cystocarp must then be regarded as sporophytic in character. Because the mitotic figures in advanced stages of the cystocarps showed certain peculiarities Wolfe concluded that a reduction of the chromosomes occurred preliminary to the development of the carpospores. However, he did not trace a process of chromosome reduction through the characteristic phase of synapsis followed by a heterotypic mitosis, and on this point of his investigation more evidence is to be desired. There are certain reasons why in life histories among the Rhodophyceæ of the type of *Nemalion* the process of chromosome reduction may be expected to occur at the time of the germination of the carpospore.

[13] Wolfe, J. J., 1904, "Cytological Studies on *Nemalion*," *Ann. of Bot.*, Vol. XVIII, p. 608, 1904.

The investigations of Yamanouchi[14] on *Polysiphonia* established the important fact that the carpospore of this form retained the diploid number of chromosomes (forty) present throughout the sporophytic tissue of the cystocarp, and gave rise to a sporeling bearing this number in its nuclei. Furthermore, the tetrasporic plants were found to be diploid in character and the presence of the period of chromosome reduction was established in the tetraspore mother-cell. The nucleus of the tetraspore mother-cell passes through a clearly defined stage of synapsis, following which the forty sporophytic chromosomes are found associated in twenty pairs (bivalent chromosomes). The first mitosis distributes the members of these pairs and is therefore a reduction division. The tetraspore has then the haploid number of chromosomes (twenty) and on germination develops a sporeling with this reduced number, which was found to be characteristic of the sexual plants. It is reasonable to conclude that in the life history of *Polysiphonia* the sexual plants alternate with the tetrasporic, each phase arising from the spores produced upon the other. Yamanouchi expressed the belief that "the sexual plants and tetrasporic plants present two distinct phases of an antithetic alternation of generations, with the cystocarp a part of the sporophytic phase".

The many interesting points in the history of the development of the sexual organs and the cystocarps are too detailed to be presented in this brief review. Yamanouchi, however, supports Wolfe's conclusion that the trichogyne in early stages of development contains a nucleus, and that the sperm is the homologue of an antheridium, although in *Polysiphonia* this cell is uninuculeate. Some criticisms of Kurssanow[15] on the work of Wolfe upon these latter points must also be omitted.

[14] Yamanouchi, S., 1906, "The Life History of *Polysiphonia*," *Bot. Gaz.*, XLII, p. 401, 1906.

[15] *Flora*, Vol. XCIX, p. 111, 1909.

A detailed study by Lewis[16] of the life history of *Griffithsia* is in agreement with the results of Yamanouchi on the essential facts of an alternation of sexual and tetrasporic plants and the behavior of the chromosomes throughout the various phases of the similar life histories. The sexual plants have seven chromosomes, the haploid number. The cystocarp presents nuclei with fourteen chromosomes and is clearly a sporophytic phase which, as in *Polysiphonia,* develops with the cooperation of the cytoplasm in certain cells of the gametophyte; the carpospores have fourteen chromosomes. The tetrasporic plants are characterized by fourteen chromosomes, the diploid number, and may be assumed to arise from the carpospores. The first mitosis in the tetraspore mother-cell is a reduction division preceded by the characteristic stage of synapsis from which the chromosomes emerge in seven pairs. Seven chromosomes, therefore, pass to the tetraspores from which the sexual plants may be expected to develop.

It is known that in a number of species of red algæ structures resembling tetraspores are occasionally found on sexual plants and that procarps are sometimes present on tetrasporic individuals. Such conditions, first observed by Bornet, have more recently been noted by Lotsy for *Chylocladia kaliformis,* Yamanouchi for *Polysiphonia violacea,* Lewis for *Griffithsia Bornetiana,* and by the writer for *Spermothamnion Turneri, Callithamnion Baileyi* and *Ceramium pedicillatum.* Furthermore, certain of the red algæ, as *Rhodymenia palmata* on the New England coast, present tetrasporic plants in great abundance, while cystocarpic individuals are rare or unknown. Certain critics see in the above phenomena serious difficulties for the theory of alternation of generations in the Rhodophyceæ so strongly supported by the work of Yamanouchi and Lewis.

These authors had only limited material of what seemed

[16] Lewis, I. F., 1909, "The Life History of *Griffithsia Bornetiana,*" *Ann. of Bot.,* Vol. XXIII, p. 639, 1909.

to be tetraspore mother-cells on sexual plants, but in this material the apparent tetrasporangia failed to mature tetraspores, the cleavage furrows proceeding only a short distance into the mother cells while the nuclei either remained undivided (*Polysiphonia*) or produced groups of several nuclei (*Griffithsia*). Thus such cytological evidence as we have on these irregularities indicates them to be abnormal developments. Examples of apogamy and apospory are now recognized as by no means uncommon in the higher groups of plants exhibiting alternation of generations, and it will not be at all surprising if such phenomena or related irregularities of life history are found among the Rhodophyceæ. Such peculiarities must of course be thoroughly studied to determine whether or not they will prove to be the exceptions that support the rule. The investigations of recent years on apogamy and apospory in the pteridophytes have in no way weakened the acknowledged alternation of generations in that group.

A very interesting problem is presented in the phenomenon described by Osterhout[17] of the germination of the tetraspores of *Agardhiella tenera* (*Rhabdonia tenera*) while still imbedded in the tissue of the parent plant. A peculiarity of this development is the behavior of the group of tetraspores as a unit, so that all four cells enter into the formation of a sporeling. It is important to note that the sporelings are very commonly sexual plants, as would be expected from the germination of normal tetraspores. It seems quite possible that the occasional tetrasporic shoots reported by this author are products of a tetraspore mother-cell in which the reduction mitoses have been suppressed, and therefore the tetrasporangium, behaving like a monospore, would naturally give rise to a tetrasporic plant. This is a problem which should be investigated, because if the above suggestion prove true, this behavior of the tetraspores of *Agardhiella* would

[17] Osterhout, W. J. V., 1896, "Life History of *Rhabdonia tenera* J. Ag.," *Ann. of Bot.*, Vol. X, p. 403, 1896.

present strong evidence in support of the theory of alternation of generations in the Rhodophyceæ. The peculiarity of *Rhodymenia palmata* on the New England coast, referred to above, may likewise rest upon a suppression of reduction phenomena in the tetrasporangium which would lead to the omission of the sexual phase from the life history and permit of an indefinite succession of tetrasporic plants.

It is very important that the life histories of some of the simpler red algæ be investigated, especially such forms as have *Chantransia*-like stages characterized by the production of monospores. The fact that the reduction divisions of *Polysiphonia* and *Griffithsia* are not associated with the formation of carpospores leads one to suspect that the period of chromosome reduction in such types as *Batrachospermum* and *Nemalion* may occur at the time of the germination of the carpospore. Should this prove to be the case, the origin of the tetrasporic phase, characteristic of the higher Rhodophyceæ, may without difficulty be conceived as the result of a postponement of the reduction divisions and their association with a type of cell similar to and perhaps identical with a monosporangium. Such a postponement or delay in the expression of the reduction divisions would establish at once a plant with the diploid number of chromosomes (tetrasporic plant) and introduce into the life cycle the tetrasporangium as a new type of reproductive organ.

Lewis regards the tetrasporic plant as illustrative of "an homologous alternation of generations, not the equivalent, wholly or in part, of the sporophyte of the archegoniates", and he thereby limits the sporophytic phase in the life history to the sporogenous cells of the cystocarp. This conclusion is based on the morphological resemblance of the tetrasporic plants to the sexual, although Lewis himself points out important differences between the two. Thus "tetrasporic plants (of *Griffithsia Bornetiana*) are always more abundant, as well as on

the average larger than sexual plants'' a condition which is true of a number of species of the Rhodophyceæ with which the writer has had a rather extensive field acquaintance. This is a point of some importance, since too great emphasis may be placed on the resemblance in form between the two generations (a resemblance which seems most natural since the plants develop under closely similar life conditions), and important differences in size, dimensions of the cells, and general vegetative vigor may not receive the attention that they deserve. If the fusion of gamete nuclei is to be regarded as the stimulus to a sporophytic generation, the period of chromosome reduction is equally characteristic of its end, and the second event follows as a natural consequence upon the first. Mitoses with the diploid number of chromosomes whenever they occur between these two events furnish, in the opinion of the writer, the only safe criteria of the extent and duration of a sporophytic generation in normal life histories.

Yet it has become clear from recent research on apogamy and apospory that the mere number of chromosomes, whether haploid or diploid, does not determine the morphology of the generation, gametophyte or sporophyte, with which they are associated. The reasonableness of this principle is apparent when regarded from another point of view. The inheritance which is responsible for the development in a type of a sporophyte generation must be carried by the sexual phase, and the potentialities of a sexual generation must be present in the sporophyte, although in normal life histories the inheritance is latent at certain stages and only becomes operative in both generations after definite periods of development have been passed. The two germ cells, eggs and spores, do not give rise to different generations because they expressly contain a single or a double set of the chromosomes characteristic of the species; the causes of their respective developments are too complicated to be expressed in such simple terms.

To the writer the differences between the egg and the spore appear to be such as may depend primarily on a greater vigor and vitality given to the egg by the fusion of the gametes, a vigor which has expressed itself in such varied morphological manifestations because of the different conditions under which the sporophyte generations have become established in the numerous phyla characterized by their presence. The conditions affecting the sporophyte are not alone those of physical environment; there are also those evolutionary factors that operate to adjust the plant, as far as is possible, to its place among other organisms. One conclusion stands out clearly among the difficulties of these problems: as in the case of sex, the sporophyte has probably arisen independently a number of times in the evolution of plants.

So we are brought to the end of our discussion of the nuclear phenomena of sexual reproduction in the algæ to the problems of the origin of the sporophyte and the relations of the sexual and asexual generations to one another, whether or not they are essentially homologous or antithetic in the alternation of generations. It is not the purpose of this symposium to consider these matters, but allusions and inferences could not be kept out where the vital connections between the subjects under consideration and these larger speculations are so close. The immediate need of such discussion is perhaps not so great for the reason that the pages of the *New Phytologist* for 1909 have presented two important papers of Lang and Blackman representing the opposite schools together with a report of a discussion in which a number of the leading British botanists took part. That the writer's sympathies are strongly with the hypothesis of antithetic alternation of generations must have been apparent from the tenor of this paper.

CAMBRIDGE, MASSACHUSETTS,
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NUCLEAR PHENOMENA OF SEXUAL REPRODUCTION IN FUNGI[1]

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In the light of recent researches the old dogma that the parasitic mode of life tends to the disappearance of sexuality has practically disappeared at least so far as the fungi are concerned. The evidence is now generally accepted that either a typical conjugation of normally differentiated gametes and their nuclei or some form of substitute for it is everywhere present.

Ernst and Schmidt on the basis of their studies on the root parasite *Rafflesia* have also recently emphasized the fact that there is no evidence in the case of seed plants that the parasitic habit tends to the disappearance of sexuality. Farmer and Digby have described a most remarkable substitute fusion in an apogamous fern and a still more striking case of substitute cell and nuclear fusions in another apogamous fern has been recently discovered in my own laboratory. It is proper under these conditions to examine anew and most critically all cases of cell fusions with reference to their accompanying nuclear phenomena and possible significance in the life cycle in question.

The study of sexuality in the fungi has also brought to light some fundamental modifications of the process of sexual fusion as found elsewhere which enlarge our conception of the nature and significance of gametic unions. It has also revealed most curious and striking substitutions for sexual fusions. I shall review briefly the most characteristic and significant of these variations from the ordinary methods of sexual reproduction. The fusion of

[1] A paper read by invitation before the Botanical Society of America, Boston, December 30, 1909.

the amœboid swarmspores to form the plasmodium of the slime moulds has never been satisfactorily accounted for, nor related to the processes either vegetative or sexual in other groups of fungi. With the increase of our knowledge of the possible variations which the sexual process may undergo it is becoming more possible to accept the conception that in some way the formation of the plasmodium may represent either an incipient or an aberrant type of gametic union in which the normal fusion of two gametes is replaced by a massing of indefinitely numerous cells.

The cytological study of the group is only just beginning and doubtless much is to be corrected in the fragmentary observations already published. Olive, Jahn and Kraenzlein, however, agree that there are evidences of nuclear fusions either just before spore formation or earlier in the formation of the fruit bodies, and that these karyogamies are followed by synapsis and reduction divisions. The figures given show that these forms have typically developed nuclei which are favorable for fixing and staining, and indicate that whatever disagreement exists as to what occurs in the few types studied may be expected to be cleared up by further work.

The older authors were loath to regard the union of the amœbæ to form a plasmodium as involving anything of a sexual nature, but it is quite possible that we may have to extend our conception of sexual fusions at least in their primitive forms to include cases of multiple cell fusions followed by vegetative growth, and finally the fusion of the nuclei in pairs. That cell and nuclear fusions may be thus widely separated is plain from the conditions in the rusts, and in the slime moulds, as in the rusts, nuclear fusion is followed shortly by the reduction divisions.

We can not yet regard the nuclear phenomena in the slime moulds as sufficiently cleared up. In the light of what has been found in other fungi there is however certainly no ground for believing that the nuclear history

in the plasmodium is as simple as the older students believed.

As directly suggestive of what may be expected in the slime moulds, we have the well-established fact of the fusing of multinucleated gametes and the subsequent pairing of all or approximately all their nuclei. Leger and others have seen that the gametes of *Sporodinia* and other zygomycetes are multinucleated, but the accounts of the subsequent processes in the zygospore can not be accepted as well founded and no one yet has been able to trace the history of these minute nuclear granules.

Stevens, however, has established the entrance of many male nuclei and their subsequent pairing with the numerous nuclei of the eggs of *Cystopus bliti* and *portulacæ* and unpublished observations made in my own laboratory have confirmed his results for *C. bliti.* I have also found such multiple fusions of about 200 nuclear pairs in the large oogonia of *Pyronema.* Claussen in a preliminary paper, while confirming the existence of the fusion of multinucleated gametes and subsequent pairing of the sexual nuclei in *Pyronema,* claims that the fusion of the pairs is not completed till they reach the ascus.

The large size of the nuclei in the ascogenous hyphæ in Claussen's preliminary figures seems to me, however, to confirm my own conclusion that the fusion is already completed at this stage. These multinucleated gametes prove convincingly that our conception of the egg and male cell must be extended to include multinucleated as well as uninucleated types. The cell at the moment of sexual union may be multinucleated as well as in its ordinary vegetative stages in the hyphæ, etc. That the nuclei fuse in pairs and not in larger numbers is further convincing evidence that they are the real bearers of the idioplasm and that the constancy of the chromosome number must be maintained by the ordinary type of doubling and subsequent reduction.

With the demonstration of the existence of coenocytic gametes the nature of the coenocyte itself becomes still

clearer, as I have elsewhere pointed out. The continuous plasma membrane enclosing a coenocyte is plainly in its relation to the other cell contents to be compared with the same structure in an uninucleated cell rather than with the aggregate of membranes which bound off a mass of tissue from its environment and the cells of the tissue from each other.

It is further most interesting to note that De Bary's conception of the male element in the oomycetes as gonoplasm, a mere unbounded portion of the contents of the antheridium, has been entirely confirmed by subsequent cytological research, and it is further proof of the superior significance of the nucleus as the carrier of the idioplasm in sexual fusions that in such forms as *Cystopus candidus*, for example, it is merely one of the several nuclei in the antheridium, and that with no definitely limited cytoplasmic unit which migrates through the conjugation tube and fertilizes the egg. The differentiation of the male gamete is not here an ordinary process of cell division, but a mere flowing out of one of the nuclei of the coenocytic antheridium.

The most striking discovery as to fusion in the fungi and the one which preceded and led the way to very many of the more important later results was the observation by Wager of paired nuclei and the subsequent fusion of these nuclei in the young basidium.

Wager was mistaken in describing a series of such fusions by pairs, but the clear account of the nuclear structures which he gave and the evidence that nuclear division occurs by a karyokinesis like that of other plants and animals showed for the first time the possibility of determining the nature of the various reproductive bodies in fungi by a more exact cytological investigation of them than had before been thought possible.

Wager brought the first proof of the existence of an endokaryogamy—the fusion of nuclei not derived from separate and independent gametes as in ordinary fertilizations, but having had a similar if not identical his-

tory in the cells from which the basidium arose. Such a process was entirely unknown before in either plants or animals.

Subsequent investigations by Dangeard, Maire, Ruhland, Nichols, myself and others have shown that in the Basidiomycetes a long series of binucleated cells precedes the formation of the basidium and the nuclear fusion in it may well have something of the value of a union of differentiated gametic nuclei.

The origin of the binucleated cells in the Basidiomycetes does not apparently result from a cell fusion at a definite point in the life cycle, as is the case in the æcidium of the rusts. Such a fusion might perhaps be expected to be effected at the origin of the carpophore, but it has been established that binucleated cells may be present in the mycelium prior to the formation of the carpophore. Series of such cells may extend back almost if not quite to the germination of the spore from which the mycelium arose.

A fusion of gametes morphologically equivalent to those of other algæ and fungi seems to have quite disappeared from the group so far as known at present, though it is to be remembered that comparatively few forms have been investigated as to the early stages of the formation of the carpophore. It seems probable that the endokarygamy in the basidium may have functionally replaced an older fusion of differentiated sex cells.

We have then on the present evidence a large group (over 9,000 species, according to Saccardo) of saprophytic and parasitic organisms in which normal sexual reproduction has been replaced by the fusion of nuclei of probably separate descent through a long series of cells, but which are contained at each stage within the limits of a single cell body.

The most thoroughly worked out modification of sexual reproduction in the fungi is the fusion of uninucleated gametes producing binucleated series of cells in which nuclear fusion follows only at a much later period. Such

fusions seem probably to be present in all rusts which have an æcidium or primary uredo and may occur in two forms involving in the one case merely the migration of a nucleus into a so-called fertile cell which then develops into the chain of æcidiospores, while in the other case there may be a complete cytoplasmic union of equivalent cells the so formed binucleated fusion cell then developing into a row of æcidiospores or a series of primary uredospores.

Dangeard and Sappin Trouffy discovered that the binucleated cells in the rusts originate with the æcidium, but the real significance of the binucleated condition and the method of its origin first became clear with the work of Blackman, Christman and Olive.

It is quite probable, as Blackman maintains, that these fusing cells in the æcidium of the rusts have been much modified from the ancestral conditions of the sex cells of the group, so that their fusion is to be properly characterized as a vegetative fertilization. Still there can be no question that functionally these are sexual unions, and we can hardly imagine anything more illuminating as to the relations of the male and female pronuclei in the cells produced by fusion and hence containing the double chromosome number. There can be no question here that the male and female chromosomes maintain their independence throughout the entire sporophytic life cycle and that nuclear fusion and synapsis are two closely associated phases of that more intimate union of the chromosomes which the behavior of hybrids suggests must occur at the close of the sporophyte. Such a series of binucleated cells is unknown among animals but the tendency to persistent independence shown by the pronuclei in the embryonic development of *Cyclops* shows that there is every reason for believing that here and probably in all nuclei with $2n$ chromosomes the hereditary idioplasms from the male and female parents maintain quite the same physical independence throughout the sporophyte as is so convincingly shown in the rusts.

The phenomena of conjugate division also indicate very clearly how the mechanism of karyokinesis, including centrosomes, asters and spindles, may be partly or wholly combined in a single system without necessitating a similar union of the idioplasmic units.

One of the most important points in this sexual process in the rusts is that the nuclei do finally fuse in the teleutospore. One might suppose that since the nuclei can function as physically independent units through the whole life of the sporophyte with its manifold vegetative and reproductive phases, a reduction division might be accomplished by the mere insertion of a wall between the conjugate nuclei. This may be what occurs in *Endophyllum* according to Maire. Still the fact that the nuclei fuse before the heterotypic division in practically all other rusts is certainly strongly suggestive that synapsis and its accompanying phases represent a stage of mutual influence if not of interchange of physical material between the chromosomes much more intimate than any which has preceded it in the life of the sporophyte.

On the other hand, it is plain that the nuclear fusion is unnecessary so far as the sporophyte itself is concerned. The sporophyte of the rusts, as in other plants, is the distinctively dominant and progressive phase in the life cycle of the fungus. The uredo mycelium and the rapid succession of crops of uredospores with all their adaptations for rapid spread and virulent development are a parallel in every respect to the sporophyte in every type of plant in which it is found from the ferns to the seed plants. It is plain then that the vigor and adaptability of the sporophyte are not dependent upon the combination of the sets of parental chromosomes in a single nucleus. The same results are possible with two more or less independent nuclei in the cell, each containing the chromosomes from one parent.

For the ascomycetes Dangeard extended Wager's observation of endokaryogamy in the basidium by the discovery of a nuclear fusion in the ascus similar to that

in the basidium and teleutospore. Dangeard pronounced these nuclei which fuse in the ascus the morphological equivalents of the gametes of other fungi and algæ and the resulting ascus a fertilized egg; a view in which he has not been followed by other students of the group. It seems probable, however, that this fusion functionally may have the value in greater or less degree of a sexual fusion, and that in cases in which the normal union of gametes has disappeared this endokaryogamy may be a substitute for it.

The most puzzling feature of the sexual reproduction in the ascomycetes and that about whose existence, I may add, there is least agreement, lies in the fact that in the course of a single life cycle we have two nuclear fusions. At the origin of the ascocarp we find the formation and fusion of normally developed sexual cells and nuclei and in the young ascus a fusion of included nuclei. There is practically no dispute at present that the gametes formed at the origin of the ascocarp represent the original and normal sex organs of the fungus and for *Pyronema* the fusion between the antheridium and trichogyne is universally admitted.

My own studies have convinced me that in the mildews and *Pyronema* at least we have a normal conjugation of differentiated gametes at the origin of the ascocarp and an endokaryogamy in the ascus.

Blackman and Fraser confirm the existence of the two fusions in *Sphærotheca* and Blackman and Fraser (1906, *Humaria granulata*), Fraser and Chambers (1907, *Aspergillus Herbariorum*), Welsford (1907, *Ascobolus*), Fraser (1908, *Humaria rutilans*), Cutting (1909, *Ascophanus*) and Dale (1909, *Aspergillus repens*) describe the occurrence of two endokaryogamies in a single life cycle.

As already noted, Claussen in a recent preliminary paper attempts to resolve this difficulty in *Pyronema* by claiming that while there is a normal cell fusion at the origin of the ascocarp the nuclei of the gametes do not

fuse, but are paired and divide by conjugate division in the ascogenous hyphæ, fusing only after they reach the young ascus. This is a direct transference to the ascomycetes of the conditions described by Blackman and Christman for the rusts, and it is certainly an obvious and easy suggestion. It was made in a letter to the writer by Raciborski in 1895 and later published by him.[1] In my opinion, as noted above, judging from the size of the nuclei in Claussen's figures of the young ascogenous hyphæ, the nuclear fusion has already occurred at this stage.

It is evident that such profound changes as have led to the wide-spread occurrence of endokaryogamy among so many and such diverse groups of the fungi can hardly have come about suddenly. Even on the mutation theory it would hardly be supposed that in the rusts, for example, the disappearance of function in the spermatia, the new fusion in the æcidium, the long series of conjugate divisions in the destructive uredo stage of the fungus and the endokaryogamy in the teleutospore should all have appeared by a single step simultaneously. There is a degree of correlation in all these changes without doubt, but they must fairly be assumed to have worked themselves out gradually in connection with the development of the complicated life history with heterœcism and the numerous spore forms which characterize the group.

It is evident that at present the fusion of nuclei in the teleutospore is simply the delayed union of the gametic nuclei that came together in the cell fusion at the base of the row of æcidiospores. In my opinion, however, as I have argued at length elsewhere, this fusion in the spore mother-cell originated as a purely vegetative union associated in the ascus and basidium with the development of the relatively gigantic size of these cells and the maintenance of the nucleo-cytoplasmic relation, the factors involved being the abundance of nutritive material concentrated in the ascus and basidium as sporebearing or-

[1] *Flora*, 1896, p. 132.

gans and their function as spore mother-cells which are to undergo reduction divisions and close the sporophyte generation.

From this standpoint it is to be remembered that we have considerable evidence that in the rusts with abbreviated life cycles the binucleated condition and conjugate division extend back from the endokaryogamy to more or less vague and uncertain points in the life cycle of the sporophyte. For example, in the short-cycled rusts with only teleutospores Blackman, Maire and Olive find that the binucleated condition extends back from the teleutospore to some undetermined point in the mycelium from which the teleutospore sorus arises. As already noted, it is certain that in various basidiomycetes the binucleated condition extends back through the carpophore to some not sharply marked stage in the mycelium. Similar conditions may be present in the smuts.

In *Pyronema* I have found that the paired nuclei in the young ascus arise by simultaneous division of two mother nuclei in the tip of the ascogenous hypha from which an ascus is to arise and that the spindles are so placed that the two nuclei which are cut off in the young ascus and subsequently fuse are not sister nuclei. This final nuclear division is similar to the conjugate nuclear divisions in the rusts. Recently Guilliermond and Maire have described cases among certain of the larger discomycetes in which the ascogenous hyphæ were found to consist of binucleated cells for some distance below the young ascus, and suggest that these are comparable to the binucleated series of cells in the rusts. In the majority of cases so far described the ascogenous hyphæ are multinucleated as in *Pyronema* and *Ascobolus*. In the mildews the cells of the ascogon below the ascus are uninucleated and it does not appear that the nuclei which fuse in the ascus come from any such distinct lines of nuclear descent as do those which fuse in the teleutospore of the rusts.

The binucleated condition may be working backward

from the endokaryogamy in the ascus in the forms noted by Guilliermond and Maire, and further we have no cases as yet in which the binucleated condition originating in a cell conjugation without nuclear fusion is working forward into the sporophyte. In the full-cycled rusts the binucleated condition and conjugate division extend from the gametic union to the reduction stage in the spore mother-cell. This is manifestly a condition of equilibrium comparable to the stable conditions present in most forms with definitely limited alternation of generations. In *Pyronema* in case Claussen's preliminary contentions should turn out to be true the same condition would be presented. We have nowhere evidence that the binucleated condition and conjugate division originated in gametic fusions and worked gradually forward through the sporophyte.

As perhaps the most striking evidence that the saprophytic habit has not led to the disappearance of sexuality we have the recent evidence as to the occurrence of sexual fusions in the yeasts. It is probably too early to speak with definiteness as to nuclear phenomena in these minute cells. Still there can be no question that in *Zygosaccharomyces* we have normal conjugation of gametes followed by two divisions and spore formation quite probably representing a reduction stage. In *Saccharomyces Ludwigii* just as clearly a double division period and endospore formation is followed by conjugation. This transposition of the sexual stage may be secondarily developed as Guilliermond believes, or may indicate the polyphyletic nature of the yeast group. There can at least be no question that sexuality either in a primitive or modified form has been maintained in this otherwise highly specialized saprophytic group.

Our knowledge of the conditions in the smuts as the result of the work of Dangeard, myself, Federley and Lutman, in a paper now in press, indicates that in this group also two fusions may occur in a single life cycle but under conditions which make the relations of the two

to each other and to the original sexual reproduction of the group much clearer. In the smuts we have an endokaryogamy in the smut spore. In *Entyloma* the mycelium from which the spore arises is binucleated at least in the later stages of its development. In *Ustilago* the mycelial cells are multinucleated and there is less evidence for the separate ancestry of the nuclei in the smut spore.

The conjugations of the conidia regarded by DeBary as sexual unions represent the only normal conjugation of gametes in the life cycle, but they are plainly only of sporadic occurrence. Certainly they are not necessary to the completion of the normal development of the smut and they may probably be assumed to be in process of disappearance. The behavior of the nuclei in these fusing conidia favors this view. Federley finds them fusing in normal fashion in the smut of salsify. Lutman finds also that the nuclei may fuse in the conjugation of the promycelial cells of the oat smut. Dangeard finds they do not fuse in the conidia of *Tilletia* and according to my own observation the same is true in the conjugation of the conidia of the anther smut, though here many of the ordinary effects of sexual fusion appear in the conjugated pairs of conidia.

Lutman shows that infection and the normal development of oat smut may occur without any such fusions, and that hence the later nuclear fusion in the smut spore can not possibly be a delayed fusion of nuclei which came together in an earlier normal cell fusion of gametes. The endokaryogamy in the smut spore appears here as a distinct process which has originated independently of the normal sexual fusion, though it may have secondarily developed sexual significance with the development of conjugate division in the mycelial cells from which the smut spores arise, and have thus made possible the entire disappearance of the original normal sexual reproduction.

From this standpoint we are justified still further in

the assumption that the appearance of binucleated cells and possibly conjugate division in the ascogenous cells of the discomycetes mentioned above indicate not the persistence of unfused but paired gametic nuclei, but a working back of conjugate division from the spore mother cell into the tissues of the sporophyte, thus giving gradually to the endokaryogamy in the ascus more and more of a sexual significance.

The evidence has accumulated in many lines that in several groups of the fungi the sexual process is followed by a longer or shorter period of development with cells containing a double chromosome number. Evidence from a direct counting of the chromosomes before and after fusion is lacking, owing to the small size of the nuclei, still there can be no reasonable doubt that the regularly binucleated cells of the rusts which form an unbroken series from the æcidium to the teleutospore contain twice the number of chromosomes present in the uninucleated mycelial cells from which the æcidium arises. As already noted, the conditions in the rusts are especially favorable for demonstrating that the sexual fusion inaugurates a period of development with cells containing 2n chromosomes and that this sporophytic stage tends to predominate in the life cycle both as to length and complexity.

It is quite evident also in advance of a knowledge of the complete cytological data that the double division involved in forming the promycelial cells is to be considered as a reduction period.

In the smuts and Basidiomycetes the limits of the gametophyte and sporophyte are not so definitely marked as in the rusts, but the binucleated phase is certainly to be regarded as sporophytic however it may be inaugurated. In the Ascomycetes the morphological relations of the mycelium and ascocarp have long been regarded as showing a parallelism with the conditions in liverworts and mosses. The fact of a triple division in the ascus in contrast with the elsewhere universally present

double division of spore mother cells has been regarded as opposed to the interpretation of the ascus as a spore mother cell. However, as I have pointed out, this triple division replacing ordinary double division is to be regarded as correlated with two nuclear fusions preceding it.

Miss Fraser has brought further interesting cytological evidence that the third division in the ascus is a reduction division which she characterizes as a brachymeiosis. The universality of the triple nuclear division in the ascus even in cases where less than eight spores are formed is certainly to be regarded as a fact of the first importance and comparable to the universality with which a double division occurs in other spore mother cells. The most striking of the peculiarities as to cell and nuclear fusions in the fungi may be summarized as follows:

1. The fusion of multinucleated gametes.
2. The male element may be a mass of gonoplasm rather than a definitely bounded cell.
3. Endokaryogamy, the fusion of nuclei not brought together by cell fusion but of more or less independent ancestry.
4. The fusion of gametes without the fusion of their nuclei; the latter reproducing by conjugate division for long series of cell generations and finally fusing just before the reduction division.
5. Fertilization by nuclear migration from a vegetative cell to an egg or fertile cell.
6. Two successive fusions in the same life cycle, a normal conjugation of gametes and later endokaryogamy.

THE POSE OF SAUROPODOUS DINOSAURS[1]

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THESE four articles discuss a question of considerable general interest. Did the huge Sauropodous dinosaurs, *Diplodocus, Brontosaurus* and their allies, walk like elephants, or crawl like crocodiles? The skeletons and casts in the larger museums of America and Europe have all been mounted straight limbed, with the body well clear of the ground. But the evidence for giving them this pose, so different from that of the generality of reptiles, although well known to those who are responsible for it, has not until recently been published. Hence it is not surprising that these reconstructions have been criticized more or less seriously, especially in Germany, and that two writers of high scientific standing—Dr. Tornier in Berlin, and Dr. Hay in Washington—have contended that these animals could not have walked upright, but must have dragged the belly on the ground as crocodiles and lizards normally do. Both writers have attempted and discussed at length the re-articulation of the skeleton in the crocodilian pose.

Dr. Tornier's argument is, briefly, that reptiles crawl

[1] "Die Rekonstruktion des Diplodocus," von O. Abel, *Abh. k. k. Zoöl.-Bot. Gesellschaft in Wien*, Vol. V., 1909–10, pp. 1–60 of separata, three plates and text figures; March 24, 1910.

"Review of some Recent Criticisms of the Restorations of Sauropod Dinosaurs Existing in the Museums of the United States, with Special Reference to that of *Diplodocus Carnegiei* in the Carnegie Museum," by Dr. W. J. Holland, AMERICAN NATURALIST, 1910, Vol. XLIV., pp. 259–283, plate I. and text figures, May, 1910.

"On the Manner of Locomotion of the Dinosaurs, Especially *Diplodocus*, with Remarks on the Origin of the Birds," by Oliver P. Hay, *Proc. Washington Acad. Sci.*, 1910, Vol. XII., pp. 1–25, pl. 1, text figs. 1–7, February 15, 1910.

"Wie war der *Diplodocus carnegii* wirklich gebaut?" von Gustav Tornier, *Sitz.-ber. Gesell. Naturf. Freunde zu Berlin*, 1909, pp. 193–209, pl. II. and 6 text figures.

while mammals walk; that *Diplodocus* is a reptile and resembles the lizards and crocodiles far more closely than it does any mammals in the details of construction of the shoulder- and hip-girdles, limbs and feet. Therefore, it should be posed like one of the larger lizards, except for the long neck, which he compares to the long-necked birds and poses in accordance. A sketch restoration and a number of diagrammatic drawings illustrate his views. The subject appears, frankly, to be somewhat outside the range of his studies, and his comparisons are not broad or thorough enough to be at all convincing.

His criticisms are very effectively and completely answered by Dr. Abel and Dr. Holland. These authors point out that while the dinosaurs were reptiles and as such their bones were constructed upon the reptilian plan, yet they form a group apart, differing from other reptiles and in many respects resembling the struthious birds; that these resemblances, especially as regards the construction of pelvis and hind limbs, leave no reasonable doubt that the typical dinosaurs walked pretty much as do the great ground birds; that the limbs of *Diplodocus* and its allies differ from the normal dinosaur type in a marked superficial and adaptive resemblance to the elephant, indicating a quadrupedal "rectigrade" mode of motion; that the skeleton articulates satisfactorily in this pose and that the attempt to articulate it in the pose of a crocodile or lizard involves either a demonstrably false interpretation of parts, or a disarticulation of the joints which proves such a position to be highly abnormal if not utterly impossible for the creature to assume.

Dr. Hay's contributions to the discussion—the article cited and an earlier one in the AMERICAN NATURALIST—are worthy of more careful consideration. Hay is a high authority on fossil vertebrata, especially upon Chelonia and fishes, and has recently devoted considerable study to the dinosaurs. He recognizes the fact that the dinosaurs, while pertaining to the class Reptilia, form a group apart, with many analogies to the birds; that many dinosaurs did walk with the body clear of the ground, and that many

lizards walk or run in this way at times. He does not deny that even the Sauropoda may have done so at times, but regards them as too massive and heavy for this to have been their normal mode of progress. But his chief protest is against the placing of the knee and elbow joints in sagittal planes (*i. e.*, bending parallel with the middle line of the body) as in mammals, instead of bending outward as in all modern reptiles. In certain points of his argument he makes out a convincing case in the reviewer's opinion; other points may be satisfactorily answered.

Dr. Hay misstates the supposed significance of the peculiar type of femur seen in *Diplodocus*. He observes:

> If the mammal-like gait of *Diplodocus* be insisted upon on the ground of straightness of the femur, it may be pointed out . . . that the femora of *Sphenodon* and of lizards, animals that creep, are straight. If it be contended that it is in the heavy-bodied animals that a straight femur is correlated with a lifting of the body from the ground during locomotion, it may be permitted to recall that the femora of *Allosaurus* and *Tyrannosaurus*, great carnivorous dinosaurs, are distinctly bent. The femora of *Trachodon* are straight, while those of *Camptosaurus* and *Laosaurus* are curved. Curvature of the femur seems therefore to have no relation to size of body or erectness of pose.

But no one, so far as the reviewer knows, has asserted that the straightness of the shaft of the femur of *Diplodocus*, considered alone, proved that the animal walked like a mammal. For among mammals there are both straight and curved femora, and a wide variety of gaits.

The argument that Dr. Hay presumably has in mind is this: That in the elephants and several other types of gigantic mammals the femur is relatively long, straight-shafted, with its articulations terminal rather than lateral, the feet short, rounded, heavily padded and capable of but limited motion, the whole limb being pillar-like and normally held straight under the body. All gigantic mammals show some degree of approach towards this type of limb; and in the Sauropoda the resemblance in form and proportions is very marked. The same type is seen in *Coryphodon, Uintatherium, Titanotherium, Arsinoitherium, Pyrotherium, Astrapotherium, Dipro-*

todon, gigantic mammals of widely diverse stocks, in *Stegosaurus* and *Triceratops* among the dinosaurs, and an approach towards it in various other groups. The modern horses, rhinoceroses, cattle and other large animals, and most of the very large extinct mammals show a distinct approach toward these proportions as compared with their smaller and more agile ancestors; so too do the gigantic *Trachodon* and *Tyrannosaurus* as compared with their smaller and more agile ancestors or relatives, *Laosaurus, Camptosaurus,* and *Allosaurus.*

On the other hand, a glance at a lizard femur shows that the straight shaft is associated here with a wholly different position of the proximal and distal articulations and of the trochanters by which the limb is moved. The distal articulations for the tibia and fibula are on the back of the femur, not on its end; the great trochanter for the hip muscles projects outwards from the shaft instead of upwards in line with it; the feet are long and the toes relatively elongate; there is very little padding.

The shaft of the femur is nearly straight in the aquatic turtles and the articulation for the lower limb is quite distal in position; but the trochanter projects upward, the limb is carried outward from the body and more or less straight. In the land turtles and in the crocodiles the femur has a strongly curved shaft, bent downward at the distal end, the limb still projecting outward from the body but flexed sharply downward at the knee. Here then are two distinct methods by which a swimming limb may be converted into a crawling limb.

The straight-shafted femur does not *per se* prove that *Diplodocus* walked in any of the various ways that mammals walk. But taken in connection with the numerous other adaptive resemblances in form and proportions of the bones of the hind limb, feet and pelvis, to the elephants and other gigantic mammals and reptiles cited, it does afford a very strong argument for asserting that *Diplodocus* walked like an elephant as to its hind limbs.

Dr. Hay's next point appears to be a strong one. He observes that if we compare the femora of such dinosaurs

as *Allosaurus, Tyrannosaurus, Trachodon* or *Camptosaurus,* admittedly erect-walking bipedal forms, with the femora of the Sauropoda, we find a great difference in the quality of the bone and the finish of the articulations.

The shaft of the former appears to be more elaborately modelled, and to consist of finer and harder bone; all the articular surfaces are smooth and they carry the conviction that the original surfaces, barring a thin layer of cartilage, are preserved; there is a definite head, separated from the shaft by a distinct neck, and nearly filling the acetabulum; and there is a definitely formed trochanter major. In the Sauropoda, on the contrary, the shaft seems to be composed of coarser bone; the articular surfaces are rough and show that they were covered by a thick layer of cartilage; the head merges imperceptibly into the supposed great trochanter and into the shaft; [it would be more accurate to state that the great trochanter is not a separate process but a rugose area marginal to the head of the femur] and the head lacks much of filling the acetabulum. In its [*sic*] low stage of differentiation the femora of the sauropods resemble greatly those of the crocodiles and are hardly above those of the lizards.

It is not clear what lizards Dr. Hay had here in mind. The larger land lizards have a much more "differentiated" femur. Even in the crocodiles the resemblance is not very close. But a much closer and more striking resemblance in the characters cited may be found if we compare the femora of large *aquatic* reptiles, ichthyosaurs, mosasaurs or plesiosaurs, or large *aquatic* mammals such as the Cetacea, with the femora of the Sauropoda. The reviewer would agree entirely with Dr. Hay that the lack of differentiation and finish in the limb bones of Sauropoda is a strong argument that they were not adapted to the habitual support of the whole weight of the body. But the evidence cited accords exactly with the theory of Owen and Cope that they were wading animals, and the limbs were designed for the support of the body in the water, with most of its weight buoyed up thereby.

Dr. Hay believes that the position of the great trochanter in the Sauropoda was well down on the shaft, as it is in the Triassic Theropoda; but he fails to give any good reason for rejecting Osborn's view that the very clearly marked rugosity around the proximal-external angle of the head is the area of attachment of the gluteal muscles.

The character of this rugosity is certainly that of an attachment for powerful muscles; its position is substantially that of the distinct process in the larger bipedal dinosaurs; while the surface where Dr. Hay would locate the attachment is a surface of smooth bone. On the other hand, the view of Marsh and Hatcher, apparently shared by Holland, that the entire proximal-external angle, including part of the rugose surface of the proximal end, similar in character to the rest of the anatomical head or articulating surface, is the great trochanter, appears to be indefensible, and Dr. Hay's arguments conclusive as against it. This view seems to have been founded on the analogy with the proboscidean femur, carried further than the facts warrant. But Dr. Hay's conclusion that the femur of the Sauropoda represents a very early stage in progressive adaptation of the limb from the primitive swimming to the walking type, is not warranted if Osborn's view as to the position of the trochanter be correct, for as Hay rightly observes, in the progressive stages of adaptation to upright carriage this attachment moves up towards the head of the bone. But the only semblance of argument that Dr. Hay offers against this view seems to be the assumption that *Diplodocus* was more primitive than its Triassic predecessors. The truth seems to be that the Sauropoda were highly specialized as regards the adaptations for upright walking, but degenerate as regards the adaptations for bearing great weight on the limbs.

As to the aquatic habitat of the Sauropoda, Dr. Hay's statement of the evidence can hardly be regarded as a fair one, although he seems to be of the opinion that the larger forms, at any rate, were secondarily aquatic.

> The ability of any large animal to walk about thus submerged must depend on its having a massive skeleton, as have the hippopotamus and the manatee. In *Diplodocus,* on the contrary, almost every conceivable device has been used to reduce the weight of the skeleton. The great vertebræ contain large and small internal cavities, while externally the processes are carved into thin plates and buttresses, and the centra are deeply excavated on each side. Moreover, as has been shown by Hatcher, the limb bones are hollow. It would seem to have been hardly

more possible for *Diplodocus* to walk about immersed in water than it would be for a man to do the same.

In a brief description of the *Brontosaurus* skeleton[2] which Dr. Hay consistently ignores, although he can hardly fail to be acquainted with it, the reviewer pointed out that there was a very marked difference in the massiveness of the upper and lower parts of the skeleton in the Sauropoda. All the bones above a line passing through the acetabulum and glenoid cavity of the scapula are very lightly constructed, and thus far Dr. Hay's statement is correct. All the bones below this line are, on the other hand, very massive, and solid or nearly so. So far as the reviewer can judge from comparison of a large series of bones, they are quite as dense and massive as the corresponding bones in the hippopotamus; and they certainly are not hollow in the sense that the bones of *Allosaurus* are hollow. All of them are cancellous towards the center, and in the femur there is an open cavity of proportionately small size in the shaft. But they are certainly far more dense than in the elephant, and wholly lack the devices for lightening the weight that are so conspicuous in the skull, cervical and dorsal vertebræ, the first few caudals, the ilium and the proximal ends of the ribs. The median and distal caudals, the ischia, pubes and limbs, the shoulder-girdle, except for the blade of the scapula, and especially the feet, must be wholly excepted from Dr. Hay's statement in regard to the lightening of the skeleton; they are certainly unusually massive in form; and while the precise degree of density of the petrified bone is not very easily compared with modern bone, yet in the reviewer's opinion they compare most nearly with the bones of aquatic animals, such as plesiosaurs and mosasaurs among the reptiles, cetaceans, pinnipeds, sirenians and hippopotami among the mammals, and are materially exceeded in density only by the sirenians.

Dr. Hay's observations in regard to the pose of bipedal dinosaurs form an interesting corollary to his views in

[2] Amer. Mus. Guide Leaflet No. —, 1905.

regard to the Sauropoda. For although admitting that they were in general bipedal and walked with the body clear of the ground, he finds himself compelled, in the logical development of his theories, to assign them a widely straddling walk. This compels him to explain the observed tracks of *Iguanodon,* which are not straddling, as being an unusual mode of progression associated with slow walking, but that if the animal had been running it would have had a wider trackway. So far as the reviewer's observations extend, the faster an animal is walking or running the more it is inclined to plant the feet close to the median line of its trackway, and the less likely to waddle—this is assuredly true of our own gait, and probably holds good with any bipedal animal. The stronger evidence derived from the numberless tracks of the Connecticut Valley Triassic, Dr. Hay would explain by supposing that these tracks were made, not by dinosaurs which we know existed, but by birds of whose existence at that time we have no evidence.

But Lull has shown that not only do many of these tracks correspond precisely and in detail with the reconstructed hind feet of carnivorous dinosaurs, but the occasional impressions of the fore feet, the pubes and the tail, correspond equally well. A disputed hypothesis in regard to the amount of straddling in their gait is hardly sufficient ground to question the accepted view that these tracks were made by dinosaurs.

It is indeed quite reasonable to suppose that ancestors of the birds, more or less closely related to the dinosaurs, were living during the Triassic. But the extreme rarity of bird remains during the whole Mesozoic, and the fact that, with the exception of *Archæopteryx,* the better known forms are highly specialized aberrant types, gives ground for supposing that the normal habitat of Mesozoic birds was such that their remains were not buried in the areas of sedimentation. They were presumably confined to the higher and drier uplands, remote from the river-deltas and coastal swamps which were the normal habitat of the heavy and bulky dinosaurs, and which the

lighter and smaller dinosaurs of the upland visited to a greater or less degree.

Dr. Hay makes considerable use in his discussion of Marsh's restoration of *Anchisaurus*, as in this reconstruction the limbs appear relatively shorter and the proportions more lizard-like than in most Theropoda. But while this is true to some extent of *Anchisaurus* and apparently of most Triassic Theropoda, it is exaggerated in Marsh's reconstruction by insertion of several additional dorsal vertebræ which are probably not warranted.[3] The restoration is a composite from two or three partial skeletons; the number of vertebræ is really uncertain. If the body be shortened to the proportions of the more completely known dinosaurs, there is less difficulty in supposing the animal to have been habitually bipedal, and bird-like in gait. The general contention that the dinosaurs evolved from a crawling lizard-like gait to a bipedal bird-like gait without passing through a quadrupedal walking mammal-like gait appears probable enough. But the Sauropoda seem to be most easily explained by the hypothesis that they acquired secondarily a quadrupedal elephantine gait, that they were at first more or less amphibious and finally exclusively aquatic waders.

Whether we state, as does von Huene, that the Sauropoda are derived from Theropoda, or, as Dr. Hay will have it, that the Theropoda are derived from Sauropoda seems to be largely a question of terms and definitions. Both are derivable from a common ancestral group, but the Sauropoda have specialized fully as much in one direction as the Theropoda in another. In fact, in the present writer's opinion, the Sauropoda are decidedly more specialized, although their specialization is in part degenerative and a re-adaptation to the aquatic environment of the remote ancestors of the Reptilia.

[3] Marsh has inserted additional dorsal vertebræ in most of his dinosaur restorations, as may be seen by comparison with the more complete skeletons which have since been mounted and described in several American museums. There are three too many in *Brontosaurus* (Riggs), five too many in *Camptosaurus* and six too many in *Triceratops* (Gilmore) and so on probably throughout. His general concept of the analogy of the group seems to have been too much lizard-like and not enough bird-like.

Dr. Holland's article is a brilliant, well-illustrated and cruelly convincing polemic in support of the accepted pose of the *Diplodocus* skeleton, but in the reviewer's opinion he does not at all do justice to the real weight of some of the arguments advanced by Hay and Tornier, especially the former. Serious exception might be taken to the positiveness of some of his assumptions, as well as to the ridicule of his opponents' views.

Holland shows by aid of a series of photographs and carefully finished drawings that the pose advocated by Dr. Tornier could not have been assumed without an entire dislocation of the important limb joints; that the pelvis of the sauropods is like "the pelves of the dinosauria in general, distinctly ornithic in type, not lacertilian nor crocodilian," that the body is deep, narrow and short as in birds, while in the crawling reptiles it is broad flattened and more elongated; that the scapula and forelimb differ in important features from those of crocodiles and lizards, and the fore limb can not be articulated in a crawling pose; that the long heavy tail affords no argument for a crawling posture; that the feet are digitigrade and not plantigrade as asserted by Tornier; that the general form and proportions of the limbs point to an elephantine pose, and that the single known footprint of a Jurassic Sauropod supports the same interpretation. (It is worthy of note that Dr. Lull has carefully examined this footprint and come to the conclusion that it was probably made under water rather than on land.) The whole article is very readable and clearly written, and would seem to close the case so far as the possibility of Tornier's reconstruction is concerned.

Dr. Abel's contribution is a careful, thorough and fair-minded consideration of the problem by a high authority upon fossil vertebrates, who has devoted a great deal of time and thought to paleontologic reconstructions. He reviews the principles governing such work, the relationships of *Diplodocus* and the data for the reconstruction, the opinions that have been held in regard to the pose and habits of the Sauropoda, and cites Tornier's argu-

ments in some detail. He then gives a careful and critical presentation of the evidence afforded by the form and relations of the different parts of the skeleton. He concludes that the generally accepted poses, as shown in the several skeletons of Sauropoda that have been mounted, and in the published restorations by Marsh, Hatcher, Holland and Osborn, are in the main correct, except that the scapula should be somewhat more vertical, the elbow directed more outward, and fore and hind foot completely digitigrade. He finds no warrant for the radical changes in pose recommended by Tornier and Hay. The evidence can not well be condensed within the limits of a review. The marked analogy to the elephants, especially in the proportions and relations of both fore and hind feet, and in the limb bones the relationship to the bipedal dinosaurs, much closer than to the crawling reptiles, the mechanical requirements for the support of a body of the size and proportions of a Sauropod dinosaur, are the chief criteria used to interpret the direct indications from the bones of the *Diplodocus* skeleton.

Dr. Abel's conclusions may be condensed from his summary as follows:

1. The animal did not crawl, but walked, with the body well clear from the ground, the knee bending forward, the elbow outward and backward, the feet digitigrade as in the elephant. In a standing position the angle at the knee was slight (15°), while the bend at the elbow was more considerable (60°). The fore feet were longer and more completely digitigrade than the hind feet; both fore and hind feet were exaxonic, the weight of the body resting chiefly on the outer digits, which were heavily padded, with rudimentary toes, while the innermost toe of the fore foot and the inner three toes of the hind foot bore large blunt claws.

2. The body was deep and narrow, strongly arched from front to back, the neck long and flexible, normally carried forward, with the head continued in the same direction.

3. There were twelve thoracic vertebræ, of which the

first is unknown; and sixteen cervicals, of which the first (pro-atlas) is unknown. The problematic bone identified by Holland as clavicle, by Nopsca as os penis, by Tornier as episternum, is the first rib.

Some of the points cited may be questioned or disproved,[1] but the main contention, that the Sauropoda were walking, not crawling animals appears to be abundantly proved.

Nevertheless, there is a great deal yet to be said on the pose and habitat of the Sauropoda, and there are certain lines of evidence which none of the authors cited have considered adequately.

In the first place, the nature and cause of the parallelism between sauropod dinosaurs and elephants has not been very clearly pointed out. The type of limb and foot structure which they show in common was first clearly defined, so far as the reviewer is aware, by the late Professor Gaudry, under the name of "rectigradism." It is a specialization directly associated with gigantic size, the limb becoming straight and pillar-like, the foot short, round, heavily padded, with toes reduced or vestigial. The movements of the limb are chiefly at the upper joints, the foot serving chiefly as pad or cushion to absorb the shock in locomotion. This is very different from the typical "digitigradism" of the dog or cat; it may be observed with modifications, in large plantigrade and unguligrade animals as well as in digitigrade forms, and a progressive approach towards it may be observed in all races of land vertebrates as they approach gigantic size. The proximal segments of the limbs tend to become longer and straighter, their articulations more terminal, the distal segments shorter, their range of movement decreasing, the toes become much shortened and vestigial, buried in an elastic pad, or, as in ungulates, with a broad horny hoof, which absorbs shock less completely but gives a firmer footing.

[1] The insertion of two additional dorsal vertebræ is certainly erroneous, and with this falls to the ground the interpretation of the "clavicle" as a rib. Positive evidence from other partially articulated skeletons is likewise available, I believe, to determine the number of cervical vertebræ.

Obviously a specialization of this kind will occur only in an animal which habitually rests its weight on the limbs, and it is necessary with increased weight, because the increase of weight varies as the mass (cube of the linear dimensions) while the increase in strength varies as the cross section (square of the linear dimensions).

This last circumstance will very clearly set a limit to the size that an animal may attain as a practical working mechanism. And here we are brought to face an unanswerable difficulty if we consider the Sauropoda as land animals. How is it that with their less perfect reptilian organization of limbs and feet they were able to attain so much larger size than has since been attained by the land mammals with their more perfected organization. Throughout the Cenozoic we see race after race of gigantic land mammals successively culminating and disappearing, each a little larger than its predecessor, each assuming the rectigrade limb as it approaches its maximum size, the Proboscideans, the latest and largest of all, and, so far as can be judged, the most perfect in mechanical organization. But the Sauropoda, away back in the Jurassic, far surpassed the largest elephants in size; and yet their joints are rough, imperfect, cartilage covered, their muscular attachments imperfectly differentiated. If the elephant is the largest size that the perfected mammalian organization permits, how is it possible that the relatively imperfect dinosaur organization could so far exceed it? And if it is not, why have none of the numerous gigantic Tertiary mammals exceeded this size?

If, indeed, we regard the Sauropoda as aquatic animals, adapted to wading, we solve this difficulty readily enough, and find also an explanation for various peculiarities in their construction which remain unexplained if they are considered as land animals. A wading animal has the greater part of its weight buoyed up by the water, and might attain a much larger size without transcending its mechanical limitations, just as the whales and some true fishes attain a much larger size than any land animal.

A second point that does not seem to have been brought out in the discussion is that the dinosaurs are distinguished from other reptiles by the relatively large size of the legs as compared with the body. In this respect they have the proportions of mammals and birds. The significance of this would seem obvious. A crocodile, turtle or lizard crawls habitually, because his legs are not large enough to carry the weight of his body. In dinosaurs, as in mammals and birds, the legs are large enough to carry the body comfortably clear of the ground and presumably served that purpose.

The nearest approach to the dinosaur proportions is seen in some of the lizards, and it is just among these that we find a tendency to lift the body from the ground, especially during running.

Another general consideration lies in the question of the primary adaptation of the dinosaurs. Dr. Hay very justly remarks that it is by no means necessary to suppose that the bird-like (bipedal) dinosaurs had passed through a mammal-like (quadrupedal) stage of evolution. Indeed, if we regard the lizard as illustrating early stages of a similar adaptation, the evidence would be just the other way; the bipedal stage in dinosaurs came first, the quadrupedal stage was a secondary adaptation. This is generally admitted as regards the quadrupedal dinosaurs of the Predentate group; the reviewer believes that it is also true of the Sauropoda, although the indications of former bipedalism are less apparent in this group. The hypothesis would serve, however, to explain several odd features in their construction—*e. g.*, the combination of everted elbow with straight knee—and would connect them more definitely with the Theropoda of the Triassic, to which they are structurally traceable, as von Huene has demonstrated.

SHORTER ARTICLES AND DISCUSSION

EVOLUTION WITHOUT ISOLATION

THIS is the title of a brief but interesting article by O. F. Cook, in the AMERICAN NATURALIST for November, 1908. My response to the same has been delayed by the pressure of other things.

I fully agree with Dr. Cook in his statement that "The choice of words is worthy of careful consideration, but words should not lead us away from the broader issue of biological facts."

We both maintain that there may be evolution without isolation; but I do not see how he can reconcile the following statements, found in the above mentioned article, with the facts of nature. "The separation of a species into two or more parts allows the parts to become different, but there is every reason to believe that evolutionary changes *of the same kind* would take place, if the species were not divided." Again: *"Isolation,* though making more species, *impedes evolution."* Does he mean that if man and the anthropoid apes had remained one freely intergenerating species, a higher degree of intelligence would have been reached than has been attained by man under the condition of isolation between him and the apes? Does he mean that the progress of the mammals, as a whole, would have been more rapid, if they had remained one constantly intergenerating species? Does he mean that, in the case of mammals, *"changes of the same kind,"* as we now see, in size, in form, in instincts, in power to live, some in the sea like the whales and the porpoises, some on the land, some on the trees, some in hand-made houses, would all have taken place, if we, the mammals, had remained one species? Would these changes have come in successive generations, in one continuously changing kaleidoscope; or would each successive generation have become increasingly complex, till the mouse could produce, not only mice, but all other mammalian forms, including the cat, the flying-squirrel and the whale?

In many places in my volume on "Evolution," published by the Carnegie Institution, I speak of isolation and selection as *controlling factors,* while growth, reproduction, heredity and

variation are classed as fundamental conditions in evolution. Some of these passages will be found on pages 29–34, 59–60, 79–80, 138. I also show that the forms of isolation and selection, that divide and guide the process of evolution, are often determined by relations between sections of the species, and may therefore be classed as autonomic. See pp. 138–39, 141–44 and 158. These autonomic forms of isolation and selection come under what Dr. Cook defines as processes of evolution, that is "processes of spontaneous change," as does also the process of reproduction with variation. If autonomic processes of isolation and selection are forms of evolution, shall we claim that natural selection, because it relates to the power of individuals and groups to meet influences in the environment, has, therefore, nothing to do with evolution, except as it retards evolution? If the power to assimilate good and abundant food helps in the process of evolution, may not the survival of those having this power in the fullest measure help in the same process? How then can Dr. Cook say, as on the second page of his article, that isolation and selection neither cause evolution, nor help it along? I can suppose that his answer might be, that, in as far as these factors have influence, the changes produced cease to be spontaneous, and, therefore cease to be evolution, as he understands evolution. But this explanation does not seem to be applicable to cases of transformation arising under isolation and selection caused by autonomic influences, for example by sexual and social instincts.

There are, it seems to me, many difficulties in the way of regarding the term evolution as applicable only to "spontaneous processes of change," unless we class all vital action, including variation and the survival of the fittest, as spontaneous, in that it arises from within, and its origin is life producing life from itself, and never life springing out of the dead environment, or out of widely different forms of life. And then we should have to meet the objection that all life is dependent on external conditions for its food, and, therefore, for continued existence, and so no vital action is spontaneous in the sense that it is independent of conditions in the environment.

Let us suppose that a little snail, clinging to a leaf is carried by a bird, from the home of the species in a valley on this island of Oahu, and dropped in an adjoining valley half a mile away, where the conditions of soil, vegetation, rainfall and tempera-

ture are the same as in the home valley. It finds a grove of the same kind of trees as that from which it came, and from its young arises a permanent colony. At the end of a few scores of years, what do we find? It has blossomed out in a new group of variations not found in the original stock, and some of the original characters have disappeared. Here is what seems to be *"a spontaneous process of change"*; and we are about to call it a genuine case of evolution, when Dr. Cook reminds us that, if this change could not have taken place without isolation, it is not spontaneous, and that it is really a case of the impeding of evolution.

In the course of time another branch colony is formed, which varies from the original stock in habits of feeding and seeking of shelter from the sun. It gradually, but spontaneously, takes to the shrubbery near the ground, and deserts the trees; and so subjects itself to a new form of selection. The change is greater than in the former case, and is undoubtedly due to spontaneous variation, with survival of the fittest under new conditions chosen for itself spontaneously; for the kind of trees, on which the original stock lived, are found in abundance all around. Shall we call the process a case of evolution, or simply the checking of evolution through isolation and selection? I am inclined to define evolution so as to include the processes of change in such cases as these.

In this same article we find statements giving still greater limitation to what the author considers the real process of evolution. In the last paragraph it is called, "The processes of spontaneous, progressive change in species." On the third page also, we read: "Divergence may be greater than evolution when changes are not progressive, but sideways, or backwards." If the change in a species must be shown to be entirely spontaneous, and also not sideways or backwards, but upward, before we can venture to speak of it as an example of evolution, this word, now so popular, will find itself badly ostracized. "Speciation" is introduced as a rival for part of the field; but I am not sure that it will show itself better fitted than the term *divergent evolution*. I am, however, willing that the fate of the two should be left to the struggle for existence, and the success of the fittest, which I regard as one of the controlling factors in the evolution of language. The statement I am unwilling to accept is that isolation and the survival of the fittest (that is species-forma-

tion), have no connection with evolution except that they may impede the process. If all organisms came from one original intergenerating stock, deriving its food from the inorganic world, and from the dead individuals of its own kind, how far could evolution have progressed without any formation of separate species? What would now become of the organic world if isolation and selection ceased and all the separate species were merged in one? When I say that there may be evolution without isolation I mean without *additional isolation*. I do not mean that the undoing of all the effects produced by vital forces making isolation complete, though the different genera occupy the same district, would be an advance step in evolution. On the contrary, I think that such an undoing would mean the crumbling of the whole fabric of the organic world.

Is not racial evolution a term that we can rightly and wisely apply to all the processes of change in organisms affecting characters that are subject to the laws of heredity and variation? May it not be applied to all changes in races and species resulting, not only from the action and reaction of members of the same species upon each other, but also from the action and reaction between individuals or groups and their environments? May not evolution be either divergent, convergent or parallel? either progressive, or retrogressive? May it not take place without any change in the environment, and in that sense be spontaneous; and may it not be due to vital action stimulated, guided, and controlled by external conditions?

HONOLULU, HAWAII. JOHN T. GULICK.

RETROACTIVE SELECTION[1]

IN his contribution to THE AMERICAN NATURALIST of July, Professor Marshall makes some statements which I wish to correct. Among other things he says:

[1] "Retroactive Selection" is a term used by me to designate the modifications of each selection by the selections which follow. Thus, selection No. 2 modifies selection No. 1 by eliminating part of the animals which selection No. 1 retained as breeders. Selection No. 3 modifies selection No. 2 by eliminating some of the animals which selection No. 2 retained, and this in turn causes a second modification in selection No. 1 by eliminating more of those originally retained. In the same way, selection No. 4 modifies selections Nos. 1, 2 and 3; selection No. 5 modifies selections Nos. 1, 2, 3 and 4; and so on.

A study of retroactive selection is a study of the successive modifications of early selections by the retroactive effect of later ones.

Mr. Redfield would say that the effects of environment are inherited.

It would require too much space to explain how this misapprehension of my position became prevalent. It is sufficient for the present purposes to say that it is a misapprehension, and that I am not taking the ground that the effects of environment are inherited—at least not in any sense in which a plain statement to that effect would imply.

What I am interested in at the present time is learning what explanation others would give of certain facts which I have dug out of records. One of these facts relates to the ages of sires in pedigrees of 2:10 trotters, or trotters in general, and as this subject has already been opened it appears a fitting one to continue.

After quoting what I had to say about sires appearing older and older the further we go back in the best pedigrees, Professor Marshall says:

> The evident conclusion from this statement is that our best horses come from an increasing popularity of *younger* sires.

The error in this statement is more in what it implies than in what it actually says. Young sires are popular and have been popular during the entire history of breeding the trotter. Many very fast trotters have come from such sires, but a consideration of those raises a collateral question which it is better to postpone to a later date. What actually occurs in the breeding of trotters may be described as follows:

Assume some thousands of animals belonging to the trotting stock as it existed in this country, say seventy-five years ago. Using concrete numbers for illustration, we will say that out of these horses one thousand stallions are selected for breeding purposes to produce the next generation. We will designate these one thousand stallions as the first generation and the first selection in a process of selection which we will follow through several generations as it has actually been employed by the breeders of trotters. These one thousand stallions were sons of sires which were, on the average, 10.4 years old at the time the sons were born. It may be added here that this 10.4 years between generations in the male line is the approximate average as it has existed at all times within the known history of the trotter. A detailed investigation of the "Trotting Register" for different periods shows that at no time have ordinary breeding operations varied from this except in the value of the decimal.

The one thousand stallions of the first generation produce the second, of which some ten thousand or more are stud colts. Out of these a second selection of one thousand stallions is made for breeding purposes to produce the third generation. The stallions of this second selection are sons of sires averaging 10.4 years of age, and the selection is based principally upon the performances of their sisters, their cousins and their aunts. Don't fail to note the fact that until very recent years, stallions were rarely selected for breeding purposes because of their own performances. Horse history is full of the assertions by breeders that stallions intended for breeding should never be raced.

Now we come to the critical point to which close attention should be given. The one thousand stallions of the second selection are not the sons of the full one thousand stallions of the first selection—one son for each sire. On the contrary, some sires in the first selection are represented by numerous sons in the second selection, while other sires in the first selection are not represented at all. In other words, about one-half of the first selection of sires is cut out by the second selection. This weeding out of the sires of the first selection is done largely *after* the sires themselves are dead, and is based upon the performances of animals other than the sires of either generation.

When we examine the sires of the first selection after the weeding-out process involved in the second selection, we find the sires eliminated by the second selection to have been principally, though not wholly, sons of young sires; and that the sires which are retained to breed on in the male line to the third generation, are principally, though not wholly, sons of old sires. The net result of cutting off part of the original selection by the second selection is that the 500 left are sons of sires averaging about 12.5 years of age.

In due course of time a third selection of one thousand stallions is made from the third generation for the purpose of producing the fourth, and they in turn are sons of sires averaging 10.4 years of age. But all of the stallions of the second selection are not represented in the third selection. In fact about one half of them are cut off, with the result that the 500 which are left in the second selection, after making the third selection, are sons of sires averaging 12.5 years. Now, when the third selection cut off part of those originally in the second selection it also cut off many lines of descent back to the first

generation. The horses which were thus cut out of the first selection by the third selection, occurring some thirty or more years later, were more largely sons of young sires than sons of old sires, with the net result that those left were sons of sires averaging 13.5 years of age.

This is the process which has been going on from generation to generation—each selection reducing the number of horses in earlier generations which are left to breed on to later generations. Chester proved some years ago that all known standard trotters were descended in the male line from one or another of no more than seventeen foundation horses. While these all stand as having been progenitors of trotters, the majority of them represent what are now extinct families, so that the trotters as now bred come in the male line from only six or eight horses.

The process set forth explains how it comes about that, in examining the pedigrees of any trotting stock, the further we go back in those pedigrees the older the sires appear, but it does not explain *why* a late selection cuts off young sires and preserves old sires of earlier generations.

That is the supreme question I am asking biologists. I have asked it in several forms before without getting a reply commensurate with what I consider the importance of the question. It is hoped that the present form, accompanied by the explanation upon which the form is based, will bring forth a genuine effort to explain *the cause* of these remarkable facts.

CASPER L. REDFIELD.

THE LOGIC OF CHANCE IN PROBLEMS OF GENETICS

THE literature at present appearing dealing with problems of genetics and evolution teems with uncertainty and inexactness in the use and misuse of the word "chance." Some definite understanding of the significance of the concept as a legitimate category of scientific reasoning seems desirable. At any mention of the word chance, some listener is sure to rise in protest with the old adage—"There is no chance, it's only your ignorance." The acceptance or the rejection of this bit of prophecy depends on the ultimate postulate of the absolute uniformity of nature. The truth of this postulate is a metaphysical question with which the ordinary student of genetics is not concerned. He is reminded of the fact that Darwin and all after him have

used the word "chance" in many ways and he demands a definition of the term which shall meet his usage without involving metaphysical postulates.

The particular problem of genetics arises within a more or less precise universe of discourse. Within it lie a broad range of biological phenomena which the student of the problem is bound to take into consideration. Without this definite universe of discourse lie a still greater range of phenomena—chemical, physical, geological, etc.—which he is bound to neglect. His problem can not be made to encompass the whole universe, simply because the multiplicity of facts regarding the universe impede rather than advance the progress of the understanding of his particular problem. His universe of discourse must be precise and relatively small to be serviceable. Within this limitable universe of discourse the observer will discover certain regularities. These will constitute for him the laws. Certain other groups of phenomena will appear seemingly without regularities. These are chances. The reduction of chance to law will consist, ordinarily, of determining more facts regarding the chances and also in so extending the boundaries of the original universe of discourse that new and theretofore unobservable regularities will appear.

In problems of genetics the occurrence of an event said to be due to chance may be ascribed to two separate but independent sets of conditions. The internal conditions are those which are entirely confined to the original universe of discourse of the problem—as for instance, the conditions of the gametes in the chance union of a Mendelian hybrid. The external conditions are those which are not centered in the immediate elements. They are such as age, strength, and the like, of the gamete producing animals. Let ϕ represent the chance occurrence of any event, and let $a, b, c, \ldots$ represent a series of internal conditions either known or unknown, and let the coefficients $A, B, C, \ldots$, etc., represent the intensive value of a, b, c in determining the result. Similarly let m, n, o represent certain external conditions and M, N, O their coefficient of intensive value. Then the formula for the chance occurrence, ϕ, of an event becomes

$$\phi = f\ (Aa, Bb, Cc \ldots Mm, Nn, Oo \ldots)$$

As far as I can find in the recent literature of genetics the concept of chance has appeared with three different uses. It is

used first to describe the degree of probability for the crossing of individuals which differ more or less from one another. In this sense one may say the chance of producing a cross varies with the relative distance of the individuals producing the gametes in the Linnean classification. The second use of chance is that concerned with the occurrence of a mutant in those theories of the origin of variation through mutation. In this sense one speaks of a mutant as a chance or sport variety. The third type of usage has grown up in the literature of Mendelian crossing and of sex determination according to the male and female producing spermatozoa of arthropods. In this third type of instances the chance is reduced to the occurrence of one of two events.

It is the purpose of the present note to apply the formula of chance as previously given to these three separate uses of the term.

1. Take, for example, the type of the first usage mentioned above—the chance of a cross between different varieties and different Linnean species. If ϕ represents the chance of a cross between any two individuals, then the occurrence of that cross is determined by a series of internal conditions *a*, *b*, *c*, etc., which rest entirely within the structure of the gamete, as for instance, the innate characters of the centrosomes or the chromosomes. These are entirely contained within the cell itself and are, therefore, independent of any external modification. It is they, in all probability, that determine the amount of cleavage of a zygot produced from the union of gametes from individuals of different species, as for instance, when the eggs of *Rana fusca* are fertilized by sperm of the *Triton*, but development stops suddenly after a few irregular cleavages. The logician would say that these internal factors represented the conditions contained within the original universe of discourse of the particular problem in question. The union is also determined by external conditions *m*, *n*, *o*, etc., which lie without the gametes, as for instance, the ripeness of the eggs, as shown by Hertwig in experiments on the sea-urchin, or by the presence of alkaline in the water, as given by Godlewski when he crossed the sea-urchin with a crinoid *Antedon*. These external conditions are those that lie without the original universe of discourse of the problem.

2. Take, as a second usage of chance, the chance production of

a mutant. Let ϕ represent again such a chance occurrence. Then ϕ is a function of a, b, c, certain difficultly understood conditions which seem to reside within particular gametes and produce a new variety, differing considerably from the parental forms. The various occurrence of mutants among domestic animals probably resulted from such internal conditions. The very light silver cat, Chinnie, made his appearance about 1878, and has been the stock ancestor from which all the line-bred silver cats have since been produced. No external factors determining the occurrence of this sport could be traced. DeVries, who, of course, emphasizes these chance occurrences of the mutants, seems to believe that the internal conditions are alone responsible for these chance variations, yet MacDougal is said to have produced mutants of the evening primrose by salt solutions. If this observation is borne out by subsequent investigation, such mutant-producing factors would be represented by m, n, o of our formula, the external conditions for the production of chance occurrence ϕ, in this case the mutant.

3. Lastly, the concept of chance is of frequent occurrence in Mendelian literature. The Mendelian formula for the production of generation F^{II} demands that the independent inheritable characters shall separate in the gametes of F^{I} and recombine according to the pure law of chance, and Mendel's work on peas bears this out to a remarkable degree of accuracy. Similarly if we believe that the sex of certain arthropods is determined by male and female producing spermatozoa, but the egg is neutral, we must assume that the determination of sex is purely a matter of the chance union of the male or the female producing spermatozoa with a neutral egg. Nor in this case is the formula of ϕ without its significance. The work on certain parthenogenetic forms shows that certain spermatozoa, those without the accessory chromosome, are degenerate and presumably non-functional. This degeneration is obviously an internal condition, a, of the formula, but since it is known that various sex ratios exist all the way from the approximate one to one of many of the mammals to the very disproportionate ratios, approaching parthenogenesis in the nematodes, it may be supposed that this internal degeneration of certain of the spermatozoa is itself the function of other and unknown variables. Again, the external conditions m, n, o, such as age, maturity, nutrition, etc., may also have an influence upon the chance occurrence of the sex-producing factor of the spermatozoa.

The formula has perhaps an advantage, therefore, in suggesting a definite usage of the concept of chance in these problems, and also of suggesting, what writers sometimes confuse, the marked difference between external and internal determining factors. It rests upon a definite logical issue. Every scientific problem arises in a more or less definite universe of discourse. The limits of this universe of discourse determine the possibility of discovering regularity among the experiences it defines. Such internal regularity gives law, its absence, chance. This definition, therefore, gives to chance a definite and precise meaning, capable of an exact usage in scientific enquiries. It does not involve the affirmation nor the denial of the ultimate uniformity of nature.

ARTHUR S. DEWING.

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NOTES AND LITERATURE

ANIMAL STRUCTURE AND HABITS

PROFESSOR R. HESSE and Professor F. Doflein have undertaken the preparation of a general work on the structure and habits of animals, of which the first volume[1] has just appeared. This volume deals in a most complete way with the animal as an independent organism and the authors reserve for subsequent treatment the consideration of animals in relation to their environment. After an introduction which deals with life, protoplasm, the cell, animals and plants, the theory of descent, etc., the subject matter of the volume follows in four books dealing with the statics and mechanics of the animal body, its metabolism, powers of reproduction and inheritance, and the nervous system and sense organs. A final section takes up the relation of the parts of the animal to the whole. As a sample of the thoroughness with which the subject is treated the contents of the first book may be taken. This part opens with an account of the shapes and movements of the unicellular animals followed by a consideration of these aspects of the multicellular forms. The skeleton in the invertebrates and vertebrates is fully described. The power of animals to float in water and in air is next considered, and the remainder of the book is given over to a presentation of locomotion proper. This includes movements by cilia and by muscles and under the head of muscular locomotion is considered locomotion by steps such as is seen in leeches, etc., by wriggling as seen in snakes, eels, etc., and by means of lever appendages. This last section includes swimming by appendages, springing, running, climbing and flying, and the last of these is discussed in relation to insects, bats and birds. The treatment is rich in examples and abounds in well-constructed diagrams and clear illustrations. The remaining sections of the book are equally full and exhaustive.

The subject matter, though often complicated, is treated in an

[1] "Tierbau und Tierleben in ihrem Zusammenhang betrachtet," von R. Hesse und F. Doflein, Band I., Der Tierkörper als selbständiger Organismus von R. Hesse. Leipzig und Berlin, B. G. Teubner, 1910, 8vo, xvii + 789 pp., 480 figs., 15 Taf.

unusually clear way and is free from unnecessary technicalities; in fact the treatment may be said to be popular in the best sense of the word. This perhaps is the reason the book has been set in Gothic instead of Roman type, a fact which will appeal to the average German reader, though perhaps not to those outside Germany. The material is thoroughly modern without, however, involving the reader in present-day disputed questions, and in some respects the volume may be looked upon as a rewriting of the ground covered by Bergmann and Leuckart's "Anatomisch-physiologisch Uebersicht des Tierreiches." To this work the author acknowledges much indebtedness and appropriately dedicates his volume to its authors. In the revival of interest in the study of form and function Professor Hesse's volume should find its place on the book shelf of every zoologist.

G. H. PARKER.

PLANT PHYSIOLOGY[1]

DR. KAUFFMAN has published a very interesting paper on the influence of various substances on the sexual and other characters of certain species of Saprolegniaceae. Thorough studies of this kind are likely to add much to our knowledge of the biology of the fungi and will eventually, no doubt, furnish a mass of data which may be of material aid in the solution of some of the fundamental problems of evolution and variation.

One notable advance made by the author is the use of pure cultures obtained from single zoospores. A very successful and apparently simple method of obtaining the single spore cultures is described. It consists in making dilutions of zoospores in sterile water and sprinkling them with a pipette on the surface of a gelatine plate where they can be located with the microscope, and when they have germinated, they may be transferred to other plates or tubes.

The author does not state how many generations of each organism were grown from the same original single zoospore culture under the same conditions. We infer from his account, however, that in most cases the transfers were made from portions of the mycelium or gemmæ and were in the nature of vegetative reproductions rather than new generations.

[1] "A Contribution to the Physiology of the Saprolegniaceæ, with Special Reference to the Variation of the Sexual Organs," C. H. Kauffman (*Annals of Botany*, No. 87, Vol. 22, p. 361, July, 1908).

We doubt whether some of the conclusions reached, in regard to the causal nature of the various chemicals used in the different cultures in producing oogonia and other changes in certain of the species studied, should be accepted without further study and more conclusive evidence. The author says: "The results so far indicated show that it is possible to produce, where hitherto they were believed to be absent, antheridial branches of the normal type known in other species of the family, and that their production is conditioned by the presence of definite inorganic salts." The fact that antheridial branches appeared quite constantly in cultures containing certain inorganic salts, does not, in our opinion, necessarily prove that their occurrence was due entirely to the action of such salts. In order to make such a conclusion justifiable, it would be necessary, at least, to carry on a very long series of cultures, covering many generations of pedigreed forms originating from different individuals of the organism under investigation, to say nothing of other possible factors which may be unknown. It seems probable from recent investigations by Miss Wakefield[2] and others that there are fertile and sterile races or strains of species which differ in no other respect from each other and apparently do not depend upon the presence of any particular inorganic salt. An organism reproduced vegetatively would also presumably tend to be more constant in its behavior and show less variation than when each new generation was produced from spores.

Our studies of pedigreed strains of many generations of *Glœosporium* and *Colletotrichum* started from single spores lead us to the conclusion that there are some other factors or forces involved in the variations which occur in different generations from single spores which have no perceivable connection, so far as yet determined, with the composition of the medium in which they grow. Apparently spontaneous variations appear in such series of generations at the most unexpected moment under apparently identical conditions of environment and culture media. We say apparently because it is probably beyond our power at present to determine much less to control all the factors concerned in the growth and behavior of an organism.

[2] Wakefield, E. M., "Die Bedingungen der Fruchtkorperbildung bei Hymenomyceten, sowie das Aufftreten fertiler und steriler Stamme bei denselben," *Naturwis. Zeitschrift fur Forst- und Landwirtschaft*, 7. Jahrgang, 1909, 11 Heft, pp. 521–551, 1909.

Some series of cultures which produce no oogonia are believed by the author to possess a distinct physiological character in this respect, whereby they can be readily separated from series which produce oogonia. If these organisms obey the same general laws as those with which we have been working, it would be injudicious, at least, to say positively that they do not produce oogonia without having grown them for many generations and from different individuals of the same origin.

The idea, that the same conditions of nutrition, temperature and general environment always produce the same results upon the individuals of a species of organism, has, we believe, as yet no sufficient basis in demonstrated fact. The suggestion of the author, that a standard for the measurement of species comparable to the standards used in physics and chemistry, can be devised, is, we fear, a delusion and indicates a failure to recognize the apparently fundamental fact of biology that organisms possess inherent tendencies or forces which may enable them to vary without necessary dependence on any particular nutritive substance or environmental condition which can be at present definitely determined. The causes of these variations or mutations may perhaps be determined when we are able to recognize, analyze, measure and control all the numerous delicate complex factors concerned, many of which are now probably beyond us and not even surmised.

At another point essentially the same solution of the ever-vexing and recurring species question is given as follows: "At first thought this seems to indicate that no separation can be made of the different species of Saprolegniaceæ by means of constant characters. We can, however, use the resources of the chemist and physicist, and, by stating the exact conditions of culture establish a standard to which all forms may be referred." It is further stated: "It would seem necessary, then, in monographing such a family as the Saprolegniaceæ to refer all the species to definite conditions, which should be uniform for all, and to determine in each case the variability to the extreme attainable limits." If it were practicable to apply such a test, there is apparently little to justify the expectation that it would prove satisfactory. That mutations or variations may be induced or brought into expression by changes in conditions or other stimuli, is probably true, but to determine among the multiplicity and obscurity of the factors in any particular case, just

what these conditions or stimuli are, is exceedingly difficult, even though we assume in the beginning that the organism under investigation has no latent hereditary possibilities of change indirectly or not at all dependent upon its immediate environment.

Our work with *Glœosporium* and *Colletotrichum*, part of which is yet unpublished, indicates that so far as these organisms are concerned, at least, no such method would prove practicable; as the same organism, so far as any morphological characters or host relations serve to identify it, will frequently behave very differently when cultures of the organism obtained from different acervuli on leaves of the same host are made. This fact might be easily explained, perhaps, by the assertion that such forms are the elementary species of DeVries and others, or races or strains, but this rather complicates than simplifies the problem. If there are numerous elementary species of the same fungus to be found on the same host without recognizable morphological differences, it is certainly questionable whether any practical taxonomic work could be founded upon such a basis. Until it can be demonstrated that an organism is entirely controlled by its external environment and nutritive conditions and all its hereditary expressed and latent characters are known, it will be hazardous to base positive conclusions upon such data. We must continually keep in mind and be prepared to make allowance for the inherent hereditary characteristics and possibilities of the organism which may possibly find expression in any particular individual or generation.

The vast and complex problems of determining the origin and cause of the form and behavior of even the simplest living organism is not perhaps to be so easily solved. The more it is studied the more difficult it appears. All exact knowledge and data in regard to these phenomena are to be welcomed, however, and the more we come to appreciate the difficulties of the subject the better able we should be to devise experiments that will yield the necessary data which may eventually reveal to our finite minds some of these profound mysteries of nature.

C. L. SHEAR.